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(21) International Application Number: PCT/EP98/08187 (22) International Filing Date: 14 December 1998 (14.12.98) (30) Priority Data: 08/990,591 15 December 1997 (15.12.97) US (71) Applicant (for all designated States except US): PEPTOR LTD. [IL/IL]; Kiryat Weizmann, 76326 Rehovot (IL). (72) Inventors; and (75) Inventors/Applicants (for US only): REISSMANN, Siegmund [DE/DE]; Werner-Seelenbinder-Strasse 14, D-07747 Jena (DE). MÜLLER, Bettina [DE/DE]; Erfurter Strasse 44, D-07743 Jena (DE). KLEINWÄCHTER, Peter [DE/DE]; Dr.-Theodor-Neubauer-Strasse 38, D-07546 Gera (DE). BESSER, Diana [DE/DE]; Jenergasse 13, D-07743 Jena (DE). (74) Agent: VOSSIUS & PARTNER; Siebertstrasse 4, D-81675 München (DE).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: BRANCHED BUILDING UNITS FOR SYNTHESIZING CYCLIC PEPTIDES		
(57) Abstract <p>The present invention relates to building units suitable for the synthesis of backbone cyclized peptides. The building units of this invention have structures of the formula (I): $X-AA_m-U-AA_p-Y$ wherein AA_m and AA_p are residues of natural or synthetic amino acids and U is $C_\alpha ON[(CH_2)_{n+1}W]$ or $\Psi(C_\alpha H_2N)[CO(CH_2)_qW]$, in which C_α is a carbon atom and is the α carbon of AA_m if $m > 0$. W is $NH-X$ or COY, and X is H, Boc, Z, Fmoc, or Alloc while Y is the hydroxyl group of a carboxylic acid moiety or a carboxylic acid protecting group. The letter m refers to an integer from 0 to 10; n is a number from 0 to 6; p is a number from 1 to 10; and q is a number from 1 to 6. The present invention is also directed to methods of synthesizing building units of formula (I) via different routes.</p>		

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BRANCHED BUILDING UNITS FOR SYNTHESIZING CYCLIC PEPTIDES

1. TECHNICAL FIELD

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The present invention relates to a method for preparing branched building units for use in the synthesis of backbone cyclized peptides, and to the building units themselves.

2. BACKGROUND OF THE INVENTION

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Biologically active peptides are conformationally restricted through cyclization to achieve metabolic stability, to increase potency, to confer or improve receptor selectivity, and to control bioavailability. The use of medium and long-range cyclization to convert natural bioactive peptides into potential peptidomimetic drugs has been prompted by the ability to control these important pharmacological characteristics. Furthermore, the structural constraints on a peptide brought about by cyclization also enhance conformational homogeneity and facilitate conformational analysis. See, e.g., Kessler, H. Angew. Chem. Int. Ed. Eng. 21:512 (1982). Thus, cyclization may give insight into the biologically active conformations of linear peptides provided that their biological activities and selectivities are maintained.

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Common modes of long-range (or global) peptide cyclization include side-chain to side-chain, end-group to end-group, and side-chain to end-group, all of which require the alteration or removal of residue side chains. See, e.g., Manesis, N.J., and Goodman, M. Org. Chem. 52:5331 (1987). Backbone cyclization, wherein a connection is made between the N^α and/or C^α atoms of a peptidic backbone, does not require such disruption of the peptide's natural structure because only the hydrogens of the peptide bond are affected. The hydrogens are replaced by ω -functionalized chains that can be interconnected, connected to residue side-chains, or connected to the ends of a peptide to form the desired cyclic peptide. Backbone cyclization can thus stabilize a peptide's bioactive conformation and protect against its enzymatic degradation without altering its side chains.

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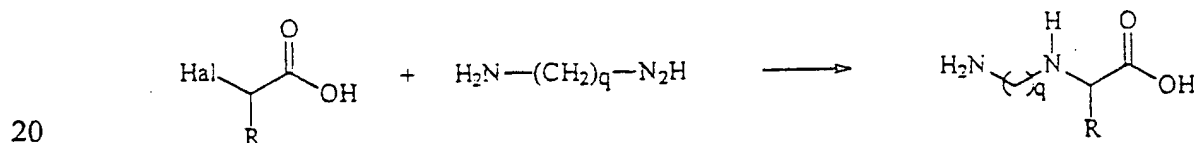
Although different methods of backbone cyclization exist, a preferred method uses dipeptide building blocks. Gilon and coworkers have disclosed backbone to side-chain and backbone to C-terminus peptide cyclization using lactam and disulfide bridges formed from such building blocks. This was done with the use of N-aminoalkyl amino acids obtained

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either by the alkylation of amino, carboxy or thiol alkyl amines with triflates of α -hydroxy acids, or by the nucleophilic substitution of alkylene diamines. U.S. Patent No. 5,723,575 and Gilon et al., *J. Org. Chem.* 57: 5687-5692 (1992) (collectively „Gilon“).

In the first method of synthesizing N-aminoalkyl amino acids, a diamine is reacted with an α bromo acid to provide an ω amine which is then selectively protected. Variation of the protecting group provides a building unit suitable for Boc chemistry peptide synthesis. In the second method of synthesizing these building units, a selectively protected diamine is reacted with chloroacetic acid to provide a protected glycine derivative suitable for Fmoc peptide synthesis.

In order to take advantage of the facile nucleophilic displacement of carboxylic acid substituents, both synthetic methods described by Gilon require the reaction of a molecule of formula Hal-CH(R)-CO-OR' (wherein Hal represents a halogen leaving group) and an amine. The amine bears an alkylidene chain that is terminated by another amine, as shown in Scheme (I):



Scheme (I)

The terminating nitrogen atom of the resulting building unit will be contained by the moiety used to form the bridging chain of a cyclized peptide.

In a molecule where R is other than hydrogen, there is a high tendency to eliminate H-Hal under basic conditions. And because the secondary amine formed by the addition reaction is a better nucleophile than the primary amine of the diamine reactant, double alkylation products may form. This side reaction reduces the yield of the method shown in Scheme (I) to such an extent that it cannot be used for the practical production of building units based on amino acids other than glycine. Gilon, however, does not suggest backbone cyclization building units having end-group moieties that are not amine, and so only provides compounds useful in backbone to side-chain and backbone to C terminus peptide cyclization.

Other workers have described alternative backbone cyclization building blocks. For example, lactam bridges have been formed with the protected building block N(CH₂COOBu')Phe, although the synthesis of bradykinin analogues using the Boc protected building block was reportedly hindered by low yields and undesired double couplings. Reissman et al. *Biomedical Peptides, Proteins & Nucleic Acids* 1:51-6 (1994). Increased efficiency was obtained, however, when the protecting group N,O-bis(trimethylsilyl)-

acetamide. The cyclic peptides made with the building block, which contain N-alkylamide
5 bonds, were reported to be unstable under acidic conditions typical of solid phase peptide
synthesis.

The synthesis of backbone cyclized peptides using glycine-based building blocks has
also been reported by Zuckermann et al. J. Am. Chem. Soc. 114: 10646-10647 (1994). This
synthetic approach, which is limited to the solid phase preparation of N-substituted glycine
10 oligomers consists of two steps: first, a resin bound secondary amine is alkylated; and
second, a side-chain is introduced into the polypeptide by nucleophilic displacement of a
halogen with an excess of a primary amine.

A more general method for the synthesis of backbone cyclized peptides is disclosed
by Kaljuste et al. Int. J. Peptide Protein Res. 43: 505-511 (1994). By utilizing amino acid
15 aldehydes, this method allows the formation of branched building units on a solid support,
but requires that both the backbone and the branching chain of the resulting cyclic peptide
contain reduced peptide bonds. The method is further limited by synthetic and storage
problems associated with amino acid aldehydes, the relatively slow rates of alkylation of the
reduced peptide bonds formed during the reaction, and the residue formed from sequence-
20 specific side reactions that can occur during the reductive alkylation of reduced peptide
bonds. Additional purification and racemization problems are also reported.

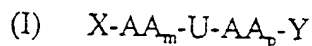
As made clear above, methods of peptide backbone cyclization have been
constrained by the limited variety of building blocks. For example, the use of similar
building block protecting groups can form hydrophobic clusters along the side chains of a
25 growing peptide which can reduce coupling rates, reaction efficiencies and yields.
Furthermore, some protecting groups can cause the racemization of terminal amino acids
during the synthesis of a cyclic peptide. A third drawback of many building blocks is that
cyclic structures formed from them degrade under the acidic and/or basic conditions typical
of solid phase synthetic methods.

30 There thus exists a need for a larger variety of building units suitable for the
synthesis of cyclic peptides, and a facile and efficient method of synthesizing such building
units. There further exists a need for building units that are stable under solid phase
synthetic conditions.

35 3. SUMMARY OF THE INVENTION

Accordingly, the present invention relates to building units suitable for the synthesis
of backbone cyclized peptides. The building units of this invention have structures of
Formula (I):

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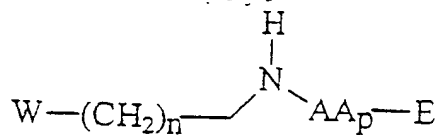


wherein AA_m and AA_p are residues of natural or synthetic amino acids. U refers to $C^\alpha ON[(CH_2)_{n+1}W]$ or $\Psi(C^\alpha H_2 N)[CO(CH_2)_q W]$, in which C^α is a carbon atom and is the α carbon of AA_m if $m > 0$. W is $NH-X$ or COY , and X is H, Boc, Z, Fmoc, or Alloc while Y is the hydroxyl group of a carboxylic acid moiety or a carboxylic acid protecting group. The letter m refers to an integer from 0 to 10; n is a number from 0 to 6; p is a number from 1 to 10; and q is a number from 1 to 6. The present invention also is directed to methods of making building units of Formula (I).

The invention more specifically is directed to a method of synthesizing building units of Formula (II):

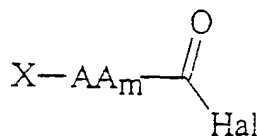


wherein n is a number from 0 to 6. According to this method, a first reaction mixture comprising a compound of Formula (d) is prepared:



Formula (d)

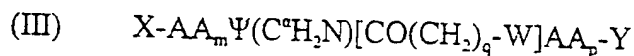
wherein E is a carboxyl protecting group. This reaction mixture is then reacted with a compound of Formula (e):



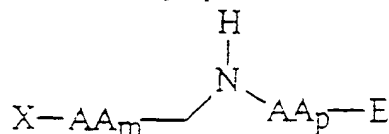
Formula (e)

wherein Hal is a halogen, under reaction conditions sufficient to form a second reaction mixture comprising the building unit of Formula (II). The building unit of Formula (II) may then be isolated from the second reaction mixture.

The present invention also encompasses a method of synthesizing a building unit of Formula (III):

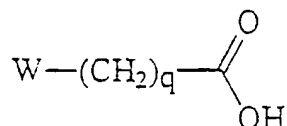


wherein q is a number from 1 to 6. According to this method, a first reaction mixture
 5 comprising a compound of Formula (m) is prepared:



Formula (m)

10 wherein E is a carboxyl protecting group. This reaction mixture is then reacted with a compound of Formula (n):



Formula (n)

15 under reaction conditions sufficient to form a second reaction mixture comprising the building unit of Formula (III). The building unit of Formula (III) may then be isolated from the second reaction mixture.

20 4. DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

All abbreviations used are in accordance with the IUPAC-IUB recommendations on Biochemical Nomenclature (J. Biol. Chem. 260:14-42 (1983)) and later supplements.

As used herein the term "amino acid" is intended to include any natural or synthetic amino acid. Abbreviations of typical amino acids used in the present invention are provided in Table (I):

25 Table (I): Amino Acids

Abbreviated Designation	Name
30 Abu	α -Amino butyric acid
Ala	L-Alanine
Arg	L-Arginine
Asn	L-Asparagine
35 Asp	L-Aspartic acid
β Asp (Ind)	β -IndolinyI aspartic acid
Cys	L-Cysteine

5	Glu	L-Glutamic acid
	Gln	L-Glutamine
	Gly	Glycine
	His	L-Histidine
	Hyp	trans-4-L-Hydroxy Proline
10	Ile	L-Isoleucine
	Leu	L-Leucine
	Lys	L-Lysine
	Met	L-Methionine
15	Nal	β -Naphthyl alanine
	Orn	Ornithine
	Phe	L-Phenylalanine
	Pro	L-Proline
	Ser	L-Serine
20	Thr	L-Threonine
	Trp	L-Tryptophane
	Tyr	L-Tyrosine
	Val	L-Valine

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Abbreviations of typical protecting groups, coupling agents, reagents and solvents suitable for use in the present invention are provided in Tables II-IV below. One skilled in the art will understand that the use assigned to each group of compounds is only a common use, and is not intended to limit the ways in which any particular compound may be
30 employed. For example, a compound listed under "reagents and solvents" may be used as a protecting group. Furthermore, it is to be understood that other possible protecting groups, coupling agents, reagents and solvents suitable for use in the present invention are intended to be within its scope.

Abbreviations of protecting groups suitable for use in the present invention are
35 provided in Table (II):

Table (II): Protecting Groups

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Abbreviated Designation	Name
Ada	Adamantane acetyl
Alloc	Allyloxycarbonyl
Allyl	Allyl ester
Boc	tert-butyloxycarbonyl
Bu ^t	tert-butyl
Bzl	Benzyl
Fmoc	Fluorenylmethyloxycarbonyl
OBzl	Benzyl ester
OEt	Ethyl ester
OMe	Methyl ester
pixyl	9-phenylxanthen-9-yl
Tos (Tosyl)	p-Toluenesulfonyl
Trt	Triphenylmethyl
Z	Benzyloxycarbonyl

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As used herein, the term „coupling agent“ refers to any compound or mixture of compounds that facilitates the coupling of two or more compounds. Abbreviations of coupling agents used herein are provided in Table (III):

Table (III): Coupling Agents

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Abbreviated Designation	Name
BOP	Benzotriazol-1-yloxytris (dimethyl-amino) phosphonium hexafluorophosphate
DIC	Diisopropylcarbodiimide
HBTU	2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
PyBrOP®	Bromotripyrrolidinophosphonium hexafluorophosphate

5	PyBOP®	Benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
	TBTU	O-(1,2-dihydro-2-oxo-1-pyridyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate

Abbreviations of reagents and solvents suitable for use herein are provided in Table (IV):

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Table (IV): Reagents and Solvents

	Abbreviated Designation	Name
15	ACN	Acetonitrile
	AcOH	Acetic acid
	Ac ₂ O	Acetic acid anhydride
	AdacOH	Adamantane acetic acid
20	Alloc-Cl	Allyloxycarbonyl chloride
	Boc ₂ O	Di-tert butyl dicarbonate
	DCM	Dichloromethane
	DMA	Dimethylacetamide
	DMF	N,N-dimethylformamide
25	DIEA	Diisopropylethylamine
	Et ₃ N	Triethylamine
	EtOAc	Ethyl acetate
	Fm	Formyl
30	FmocOSu	9-fluorenylmethyloxy carbonyl N-hydroxysuccinimide ester
	HOBT	1-Hydroxybenzotriazole
	HF	Hydrofluoric acid
	Me	Methyl
35	MeOH	Methanol
	Mes (Mesyl)	Methanesulfonyl
	NMP	1-methyl-2-pyrrolidinone

	nin.	Ninhydrin
5	i-PrOH	Iso-propanol
	Pip	Piperidine
	PP	4-pyrrolidinopyridine
	Pyr	Pyridine
10	TEA	Triethylamine
	TFA	Trifluoroacetic acid
	THF	Tetrahydrofuran
	Triflate (Trf)	Trifluoromethanesulfonyl
15	Trf ₂ O	Trifluoromethanesulfonic acid anhydride

As used herein, the term „reaction conditions“ refers to conditions under which a chemical reaction may occur, and includes, for example, temperature, time, pressure, catalysts, co-catalysts, solvent, and other variables known to those skilled in the art.

20 As used herein, the terms „building unit,“ „building block,“ „peptide building unit,“ and „peptide building block“ refer to a protected or unprotected compound that contains a moiety which may be incorporated into a peptide or peptide mimetic.

As used here, the terms "stable compound" or "stable structure" refer to a compound that does not undergo substantial decomposition under peptide synthesis conditions.

25 Because many of the compounds described herein have asymmetric centers, all chiral, diastereomeric, and racemic forms of them are included in the present invention. Many geometric isomers of olefins and the like can also be present in the compounds described herein, and all such stable isomers are contemplated by the present invention.

When any variable, such as X or Y, occurs more than once in any formula herein, its 30 definition in each occurrence is independent of its definition at every other occurrence.

The present invention encompasses building units that enable the backbone cyclization of peptides. Unlike those of the prior art, the building units of this invention typically comprise carboxyl and/or an amino groups sterically hindered only by a branching moiety. In this way, the slow coupling rates typical of prior art building blocks containing, 35 for example, N-alkyl groups may be avoided. An additional advantage of the building units of this invention is that they are easily purified via conventional extraction, precipitation and flash chromatographic means. Furthermore, the building units of the present invention are stable under both acidic and basic conditions, as well as in trimethylsilyl bromide solutions.

The branched peptide building units of the present invention are preferably formed by one of two methods: the acylation of N-aminoalkyl- or N-carboxyalkyl amino acids, or the acylation of protected pseudopeptides comprising reduced peptide bonds. In general, the individual procedures used to construct the branched peptide building units of this invention rely upon known principles of peptide synthesis, and upon solid phase peptide synthesis in particular. See, e.g., Bodanszky, Principles of Peptide Synthesis (Springer-Verlag: 1984); Bodanszky et al. The Practice of Peptide Synthesis (Springer-Verlag: 1984); Barany and Merrifield The Peptides: Analysis, Synthesis and Biology Vol. 2, Chapter 1 (Academic Press: 1980); Atherton et al. Bioorg. Chem Vol. 8 (1979). For example, synthesis of the compounds of this invention may utilize coupling agents such as those listed above. More novel coupling agents, such as pre-formed urethane-protected N-carboxy anhydrides (UNCA's) and pre-formed acyl fluorides, may also be used. Coupling reactions may occur at room temperature or at elevated temperatures, and in solvents such as, but not limited to, those listed above.

It will be appreciated that in order to achieve good yields of the compounds of this invention, it is generally necessary to protect reactive moieties during synthesis. Suitable protecting groups include those listed above, variations thereof, and additional groups known to those skilled in the art. For example, carboxylic acid end-groups may be protected by the formation of alkyl esters, substituted alkyl esters, thio esters, aryl or substituted aryl esters. As is well known to those skilled in the art, the selection of particular protecting groups used in a synthesis depends on a variety of factors, such as reaction conditions and whether or not other protecting groups are used at the same time.

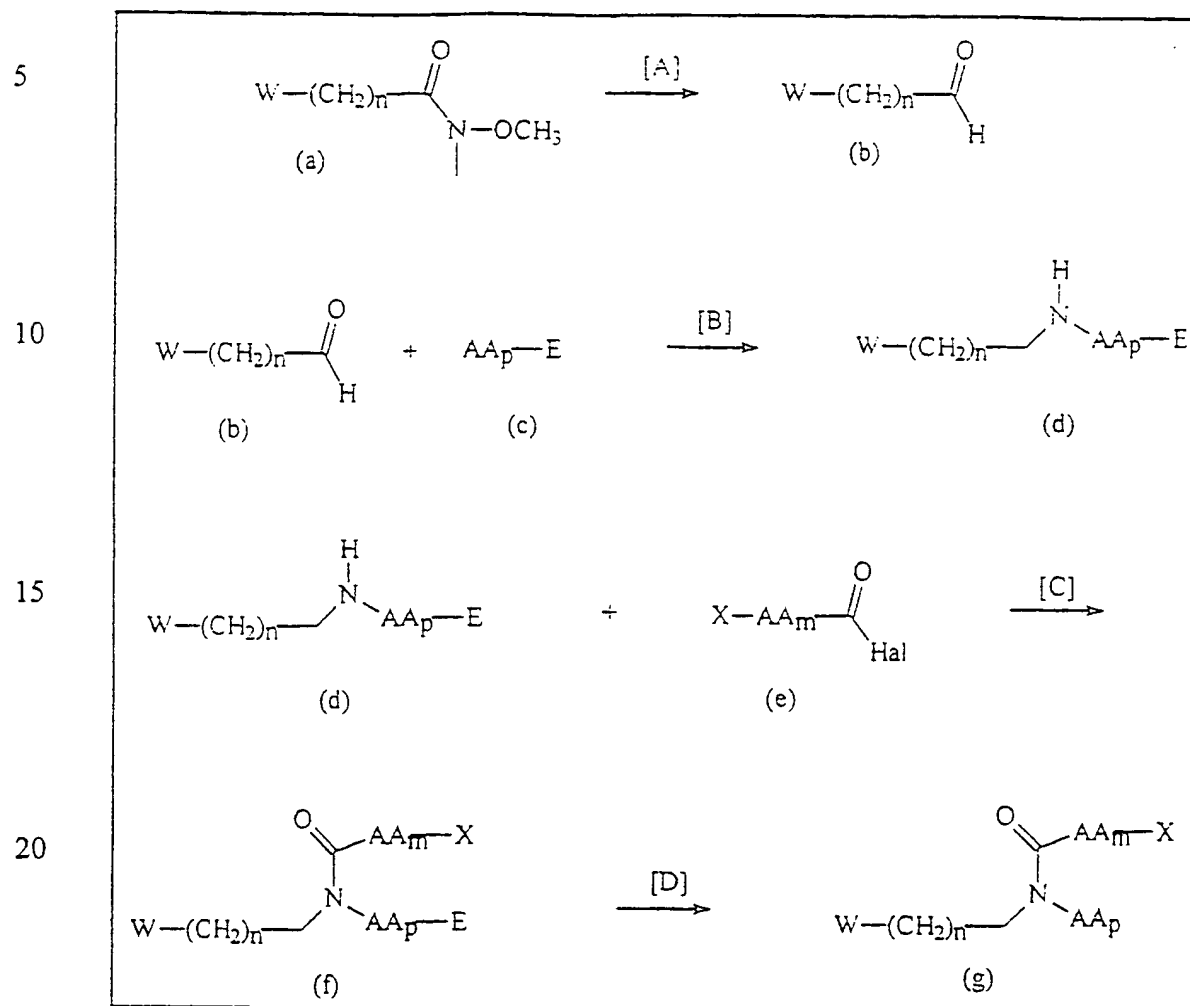
In the compounds of the present invention, AA_m and AA_p preferably designate residues of naturally occurring or synthetic proteinogenic and non-proteinogenic amino acids. Proteinogenic amino acid residues are those amino acids capable of forming proteins, and include Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr, Val, and derivatives thereof. Although these amino acids may exist in either their L- or D- configurations, the L-configuration is preferred. Additionally, non-proteinogenic amino acid residues may be incorporated into branched building units. Although these amino acid residues cannot form proteins on their own, their incorporation into a protein may, for example, aid in determining that protein's active conformers. Reporter groups such as fluorescent amino acids such as 5-hydroxytryptophan, dansyl-lysine, and their derivatives, photolabile amino acid residues, and isotopically labeled amino acids have been used for such purposes. Although these and other non-proteinogenic amino acids may be used in the present invention, AA_m and AA_p are preferably proteinogenic amino acids. AA_m

and AA_p can consist of the same or different types of amino acids. The letter m designates an integer of 0 to 10, preferably 0 to 4, and most preferably 0 or 1, while the letter p designates an integer of 1 to 10, preferably 1 to 4, and most preferably 1 or 2.

The branched building units of this invention possess protected or unprotected amide or carboxylic acid groups. These may be represented by $X-AA_m$, AA_p-Y , $NH-X$ or COY . X represents an amine or amide hydrogen when a moiety such as the N terminus of the terminal AA_m group or the artificial N terminus formed by U when $m = 0$ is unprotected. Similarly, when a carboxylic acid moiety is unprotected, Y represents the hydroxyl group of the acid. When the amine or amide to which X is attached is protected, X represents a suitable protecting group such as, but not limited to, Boc, Z, Fmoc or Alloc. When a carboxylic acid moiety is protected, Y represents a suitable protecting group such as, but not limited to, alkoxy, substituted alkoxy, allyloxy, substituted allyloxy, aryloxy, and trimethylsilyl ethers; specific examples include OMe, OBzl, OFm, OAll, and OBu^t. These protecting groups may be covalently attached to an insoluble polymeric support.

The moiety represented by U in Formula (I) refers to either $C^aON[(CH_2)_{n+1}W]$ or $\Psi(C^aH_2N)[CO(CH_2)_qW]$, wherein W is $NH-X$ or COY . C^a represents a carbon atom, and the α carbon of AA_m if $m > 0$; $\Psi(C^aH_2N)$ represents a reduced carbon-nitrogen bond, and a reduced peptide bond between adjacent AA_m and AA_p residues if $m > 0$. The letter n designates an integer from 1 to 6, preferably an integer from 1 to 4 and the letter q also designates an integer from 1 to 6, preferably an integer from 1 to 4.

The building units of Formula (II) are preferably branched dipeptides. Although these dipeptides may be synthesized by any means known to those skilled in the art, they are preferably synthesized by an approach wherein an amino acid ester is reductively alkylated to yield a N-aminoalkyl- or N-carboxyalkyl amino acid, which is subsequently coupled with a halogenated amino acid. A preferred embodiment of such an approach is shown in Scheme (II):



Scheme (II)

25 This approach reflects the inventors' discovery that benzyl and tertiary butyl protecting groups can form hydrophobic clusters that restrict the variety of solvents and reactants that may be used to form the building blocks of the present invention.

The reaction conditions represented in Scheme (II) by [A], [B], [C] and [D] may be altered or changed in ways understood by those skilled in the art, and will vary depending upon the particular reactants used and whether the reactions are performed in the liquid or solid phase. Preferably, however, the conditions are as follows: [A] represents reductive conditions suitable for the formation of a compound of Formula (b), and includes the use of, for example, (iso-Bu)₂AlH, LiAlH(O-Bu)₃, LiAlH₄-EtOH, NaAlH₄, and diaminoluminum hydrides; [B] represents conditions suitable for the reductive alkylation of the compound of Formula (c), and includes the use of catalysts such as H₂/Ni, NaCNBH₃, and BrCH₂COOBu¹/Ag₂O; [C] represents conditions suitable for the coupling of the compounds of Formulas (d) and (e), and includes the use of a coupling agent or catalyst such as collidine, BTSA, and BTSA in combination with DIEA; and [D] represents conditions

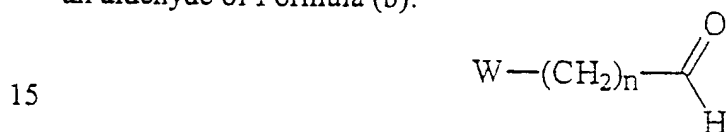
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suitable for the deprotection of the compound of Formula (f), and includes the use of an acid
 5 such as acetic acid, hydrochloric acid, and sulfuric acid in the presence of a catalyst such as palladium acetate.

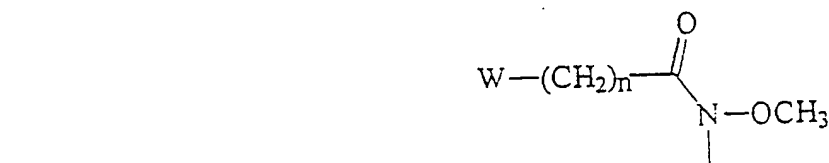
Although the method shown in Scheme (II) is a preferred method of forming
 building blocks of Formula (I), it is to be understood that variations of this method are
 encompassed within the present invention. For example, the intermediate of Formula (d)
 10 may be deprotected prior its coupling with the compound of Formula (e).

As shown in Scheme (II), an amino acid ester of Formula (c), which is preferably a
 trimethyl silyl or tertiary butyl ester of an N-alkyl amino acid, is reductively alkylated with
 an aldehyde of Formula (b):



Formula (b)

wherein W represents a protected end-group, preferably a protected amide or carboxylic
 acid, and the protecting moiety is preferably Alloc or OAll. The aldehyde of Formula (b) is
 20 preferably, but not necessarily, obtained via the reduction of an N-alkoxyamide. A preferred
 N-alkoxyamide is the N-methoxyamide of Formula (a):



Formula (a)

Reaction conditions [A] suitable for such a reduction are known to those skilled in the art,
 and include the use of reductants such (iso-Bu)₂AlH, LiAlH(O-Bu')₃, LiAlH₄-EtOH,
 NaAlH₄, and diaminoluminum hydrides, although LiAlH₄-EtOH is preferred. After much
 investigation, the present inventors have learned that the Alloc and OAll protecting groups
 30 are not readily reduced under such conditions, nor are they readily reduced during the
 following reductive alkylation reaction.

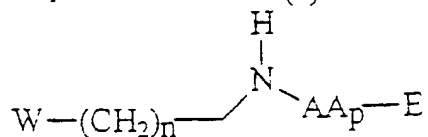
Next, according to Scheme (II), the aldehyde of Formula (b) is reacted with a
 protected amino acid or peptide ester of Formula (c):



Formula (c)

wherein E represents a carboxyl protecting group such as alkoxy, substituted alkoxy,
 allyloxy, substituted allyloxy, aryloxy, or trimethylsilyl ether, any of which may be

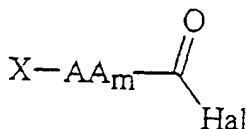
covalently attached to an insoluble polymeric support. Preferred protecting groups include
 5 OMe, OBzl, OFm, OAll, and OBu^t. This reaction is run under reaction conditions [B] that enable the formation of a compound of Formula (d):



10 Formula (d)

The reaction is preferably run in one of the above disclosed solvents, although DMF is most preferred, and a catalyst is used. Suitable catalysts are known to those skilled in the art and include, for example H₂/Ni, NaCNBH₃, and (BrCH₂COOBu^t)/Ag₂O. Preferably, the catalyst is NaCNBH₃. After the catalyst is added, the solution is stirred until the reaction is
 15 complete; a duration of 24 hours at room temperature is typically sufficient. The protected N-alkylene amino acid product of Formula (d) is then isolated from the reaction mixture using conventional extraction techniques.

The N-alkylene amino acid product of Formula (d) is then coupled with a protected amino acid halide of Formula (e):



20 Formula (e)

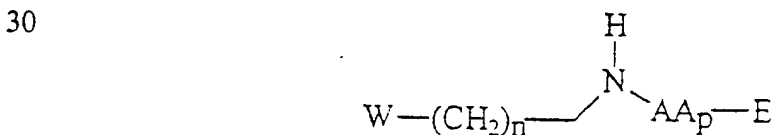
If Hal is Cl, it is preferred that the reaction conditions represented by [C] in Scheme (II) include the use of DCM as the solvent, and a coupling agent such as one of those disclosed
 25 above. It is preferred, however, that the reaction be catalyzed by BTSA alone or in combination with DIEA. If this combination is used, it is further preferred that BTSA is added to a solution of the N-alkylene amino acid Formula (d) at about room temperature over the course of about 24 hours. The solution is preferably then cooled to about 0 °C, at which point DIEA is added. At this time, the protected amino acid chloride of Formula (e)
 30 is added to the solution while the solution is stirred vigorously. Preferably, the protected amino acid chloride is added to the solution in five equal portions. Stirring is continued at about 0 °C for about 1 hour, and continues after the solution temperature is raised to about room temperature for about another 24 hours or at least until some of the dipeptide building units having Formula (f) are formed. The branched dipeptide building unit may then
 35 deprotected prior to isolation and purification, simply isolated and purified, or isolated and purified and then deprotected to yield building blocks of the present invention. It should be

noted that Formula (II) encompass both protected compounds of Formula (f) and
 5 deprotected compounds of Formula (g).

As indicated by Scheme (II), the compound of Formula (e) need not be a protected amino acid chloride. Indeed, the present inventors have learned that the N-terminal amino acid of the protected dipeptide of Formula (f) can be racemized when the reactant of Formula (e) is an acid chloride. The present inventors have also found, however, that this
 10 problem may be avoided if the compound of Formula (e) is a protected amino acid fluoride (i.e., Hal = F). Specifically, it has been discovered that dipeptides of Formula (f) may be obtained in good yield and with a minimum of racemization from the reaction of a Fmoc-AA-fluoride with an N-alkyl amino acid tertiary butyl ester. Without being limited by theory, this is believed to result from the enhanced stabilities of carboxylic acid fluorides as
 15 compared to their corresponding chlorides. Furthermore, because the N-alkyl amino acid ester of Formula (c) is more nucleophilic than the free carboxylic group, the fluoride compounds are activated to an extent sufficient to provide good coupling rates. Because different carboxylic acid halides react differently, the reaction conditions represented by [C] in Scheme (II) are preferably different than those described above for Hal = Cl.
 20 Specifically, BTSA is preferably replaced with the weaker base collidine, which is less likely to lead to the formation of undesired side-products.

The coupling reaction yields a protected dipeptide building block of Formula (f) which may be deprotected by conventional means. Specifically, reaction conditions represented by [D] in Scheme (II) include those that hydrolyze esters. For example, an
 25 organic acid such as acetic acid or a mineral acids such as hydrochloric acid and sulfuric acid may be used in the presence of a catalyst such as palladium acetate to remove the protecting group Y.

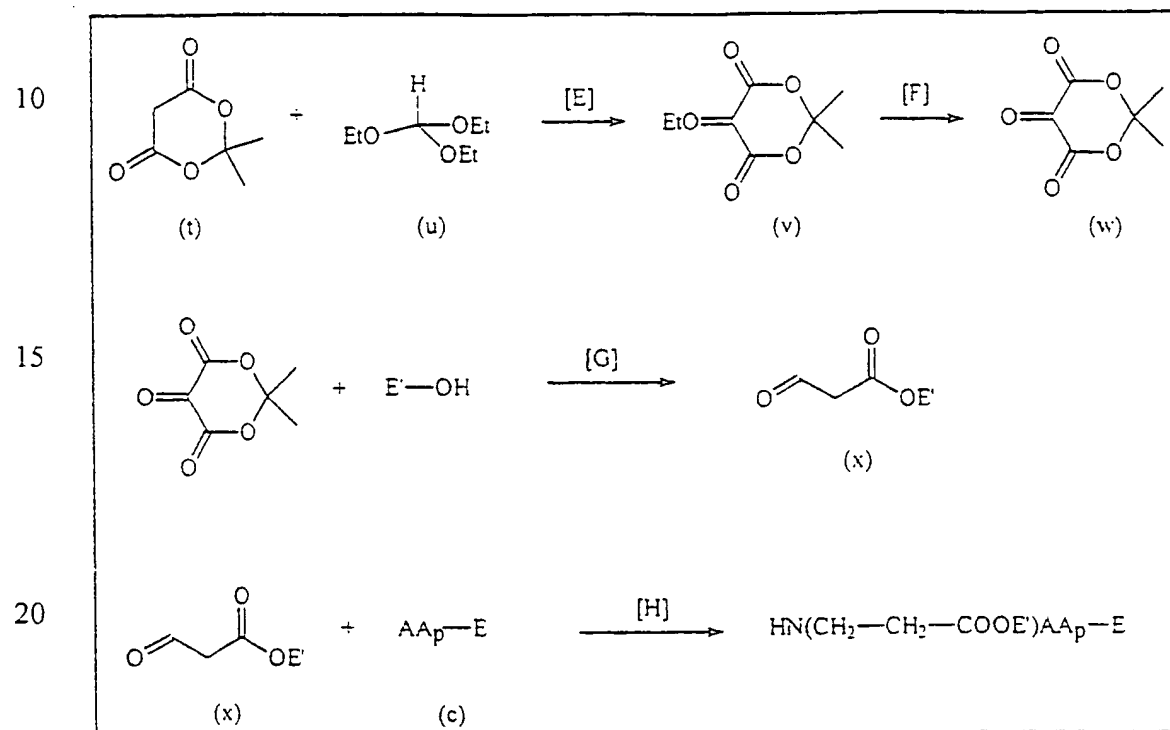
As described above, the building blocks of Formula (II) are preferably synthesized from N-aminoalkyl or N-carboxyalkyl amino acids of Formula (d):



Formula (d)

wherein W is HNX or COY. N-carboxymethyl amino acids may thus be used in the
 35 synthesis of the building units of this invention, although N-carboxymethyl amino acids tend to form dioxopiperazines under reaction conditions designated by [C] in Scheme (II). The present inventors have unexpectedly found, however, that the simple extension of the alkyl chain from methyl to ethyl avoids this problem. Accordingly, when Formula (d)

represents a N-carboxyalkyl amino acid, it is preferred that it not be a N-carboxymethyl amino acid. It is more preferred that it be a N-carboxyethyl amino acid, which may be synthesized by means other than those shown in Scheme (II). A method of preparing N-aminoalkyl amino acids of Formula (d) that has been found particularly useful in the present



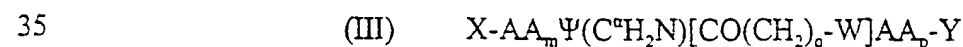
Scheme (III)

invention utilizes Formyl-Meldrum's acid as shown in Scheme (III):

25 wherein E and E' are carboxyl protecting groups. Suitable carboxyl protecting groups include, but are not limited to, alkoxy, substituted alkoxy, allyloxy, substituted allyloxy, aryloxy, and trimethylsilyl ethers; specific examples include OMe, OBzl, OFm, OAll, and OBu^t.

30

In another embodiment, the present invention encompasses peptide building units of Formula (III):



These compounds differ from those of Formula (II) in that they contain a reduced peptide bond between adjacent AA_m and AA_p residues when m > 0, and an amide bond to the

branching moiety. The reduced peptide bond is indicated by $\Psi(C^{\alpha}H_2N)$. Building units of
 5 Formula (III) allow more combinations of different protecting groups than those of Formula (II), yet exhibit similar stability in acidic and basic environments.

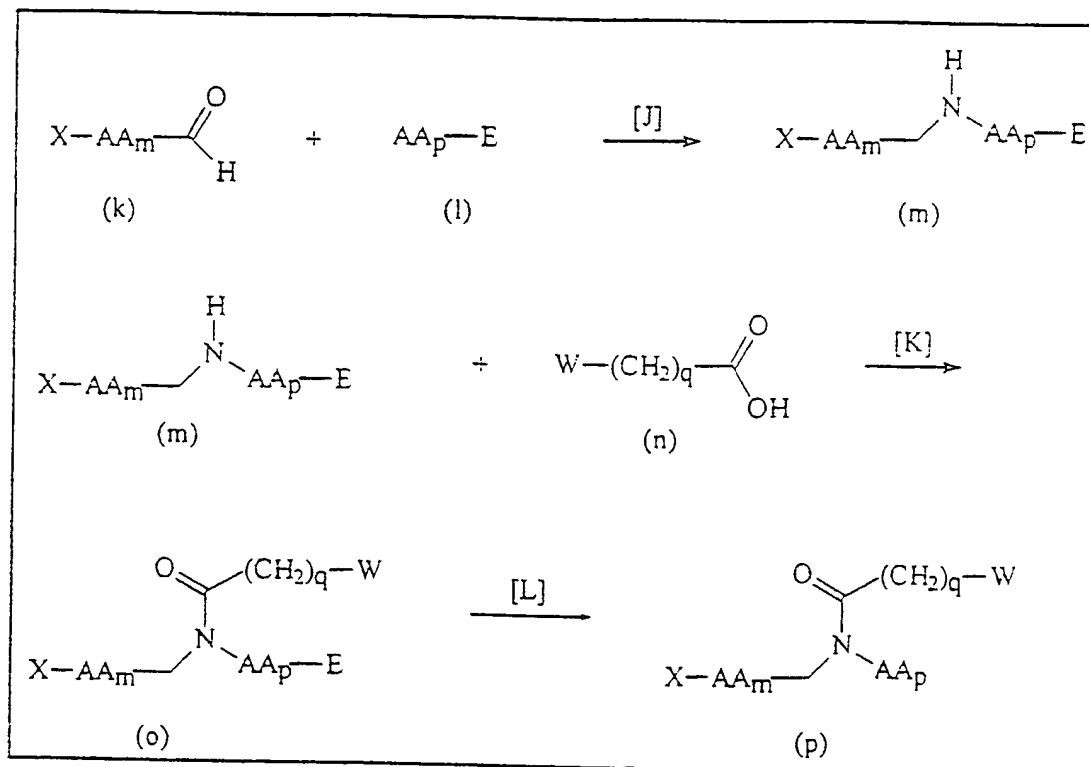
Although building units of Formula (III) may be synthesized by any means known to those skilled in the art, it is preferred that they be synthesized according to the method shown in Scheme (IV). Advantages of this method include high yields and limited

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Scheme (IV)

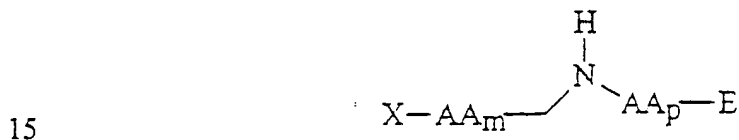
formation of undesirable side-products such as dioxopiperazines.

The approach shown in Scheme (IV) is based on an understanding of how aldehyde derivatives of natural and synthetic amino acids may be used to form dipeptides comprising
 30 reduced peptide bonds. This approach, like that shown for the first embodiment of the invention, is also based upon the inventors' discovery of novel ways by which aldehydes may be synthesized from protected amino acids, and from Fmoc-protected amino acids in particular. The synthesis of compounds of Formula (k) is thus preferably the same as that of Formula (b) shown in Scheme (II).

35 The reaction conditions represented in part by [J], [K] and [L] may be altered or changed in ways understood by those skilled in the art, and will vary depending upon the particular reactants used and whether the reactions are performed in the liquid or solid phase. Preferably, however, the conditions are as follows: [J] represents reductive

conditions suitable for the formation of a compound of Formula (m), and includes the use
 5 of, for example, catalysts such as H_2/Ni , $NaCNBH_3$, and $BrCH_2COOBu^t/Ag_2O$; [K]
 represents conditions suitable for the coupling of the compounds of Formulas (m) and (n),
 and includes the use of a coupling agent or catalyst such as collidine, BTSA, and BTSA in
 combination with DIEA; and [L] represents conditions suitable for the deprotection of the
 compound of Formula (o), and includes the use of an acid such as acetic acid, hydrochloric
 10 acid, or sulfuric acid in the presence of a catalyst such as palladium acetate.

According to Scheme (IV), an amino acid aldehyde of Formula (k) is coupled with a
 protected amino acid of Formula (l) to yield a pseudopeptide of Formula (m):

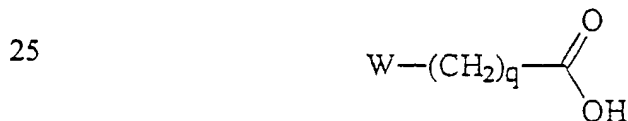


Formula (m)

wherein E is a carboxyl protecting group. Suitable carboxyl protecting groups include, but
 are not limited to, alkoxy, substituted alkoxy, allyloxy, substituted allyloxy, aryloxy, and
 trimethylsilyl ethers; specific examples include OMe, OBzl, OFm, OAll, and OBu^t.

20 Preferably, the reaction conditions represented by [J] include the use of a catalyst such as
 H_2/Ni , $NaCNBH_3$, or $BrCH_2COOBu^t/Ag_2O$, although other catalysts may of course be used.
 It has been found that the reaction proceeds smoothly if the solvent is DMF.

The compound of Formula (m) is then coupled with a carboxylic acid derivative of



Formula (n)

Formula (n):

under reaction conditions represented by [K] in Scheme (IV). Preferably, this reaction is
 30 done in DMF with the aid of a coupling agent added after the reactant mixture has been
 cooled to about 0°C. Suitable coupling agents which may then be added include, for
 example, collidine, HBTU, HOBT, BTSA, and BTSA in combination with DIEA. After all
 the reactants have been combined, the resulting solution is preferably stirred for about one
 hour at 0°C and then overnight at room temperature or at least until branched peptides of
 35 Formula (o) are formed. Because the product of this above reaction is crude, it is preferred
 that the reaction products are recoupled with another coupling agent, preferably PyBrOP®.

The reaction product may be extracted from the solution using, for example, crystallization, precipitation, or flash chromatographic means. If desired, the reaction product is then dissolved in acid, preferably acetic acid, along with a hydrolyzation catalyst such as palladium acetate to form deprotected branched dipeptides of Formula (p).

The building units of Formula (I), which encompasses those of Formulas (II) and (p), may be used in conventional solid phase peptide synthesis, and thus may be bound to other amino acids of an overall peptide structure. The coupling rates of these reactions may be monitored using quantitative tests for free amine groups and by the removal of UV detectable amino protecting groups.

5. EXAMPLES

The invention is further defined by reference to the following nonlimiting examples. The compounds described in these examples were analyzed using a variety of techniques including thin layer chromatography (TLC), high pressure liquid chromatography (HPLC), and fast atomic bombardment mass spectroscopy (FAB-MS). Thin layer chromatography was conducted using silica gel 60, F254, Merck 0.25 mm glass plates using the following solvent systems: S_1 = hexane-ethyl acetate 1/1 v/v; S_2 = chloroform-methanol 7/3 v/v; S_3 = chloroform-methanol 9/1 v/v; S_4 = chloroform-methanol-acetic acid 90/8/2 v/v/v; S_5 = n-butanol-acetic acid-water 4/1/1 v/v/v; S_6 = benzene-acetone-acetic acid 27/10/0.5 v/v/v. HPLC measurements utilized a Knauer Column (220 nm, HD-SIL-18-5s-80A, 250x4 mm) with a gradient that began at from 20% acetonitrile in 0.1% aqueous TFA and progressed to 80% acetonitrile at a rate of 1%/min; a flow rate of 1.0 ml/min was used. These examples are representative, and should not be construed to limit the scope of the present invention.

Example 1: H-N(CH₂COOBu^t)Phe-OMe

H-Phe-OMe-HCl (2.16 g/ 10 mmol) was dissolved in 50 ml freshly distilled DMF. Tertiary butyl bromoacetate (2.44 g/ 12.5 mmol) and silver(I)oxide (5.79 g/ 25 mmol) were added under stirring at room temperature. Stirring was continued for 24 hours during which the color of the silver oxide changed from black to grey. The reaction process was monitored by TLC (system 1/ ninhydrine): the starting material H-Phe-OMe-HCl disappeared as a new, main spot was detected. After reaction completion, the mixture was filtered through a silica gel bed to remove the silver salts. The precipitate was washed with DMF (3x10 ml) and the filtrates were evaporated to dryness under vacuum. The residue was dissolved in ethyl acetate (50 ml) and extracted with 5% KHSO₄ (3x10 ml), 10% NaHCO₃ (3x10 ml) and saturated aqueous NaCl. After drying with anhydrous Na₂SO₄ the

solvent was removed under vacuum. The resulting oil (2.12 g/ 72% yield) was pure by TLC and HPLC. No H-Phe-OMe-HCl was detected (HPLC t_R 5.10 min) and no diketopiperazine formation was observed. After acetylation with Ac_2O / DIPEA and saponification by LiOH/ THF at 25C°, the spot (TLC) of the product disappears within 90 minutes.

Yield: 2.12 g (72%)
 $C_{16}H_{23}NO_4$ M.W. = 293.3
 $[\alpha]_D = n.d.$
TLC Results: R_f :(S1) 0.68 (benzene:acetone:acetic acid 27:10:0.5)
 R_f :(S2) 0.82 (chloroform:methanol/ 9:1)
 R_f :(S3) 0.56 (hexane:ethyl acetate/ 1:1)
HPLC Results: t_R 17.54 min
EI-MS: $m/e[MH^+]$ 294.3

Example 2: Fmoc-D-Phe- Ψ [CO-N(CH₂COOBu')Phe-OH]

A mixture of HN(CH₂COOBu')Phe-OH (0.279 g, 1.0 mmol) in DCM (10 ml) was treated with BTSA (0.98 ml, 4 mmol) at room temperature for 24 hours. The clear solution was cooled to 0 °C and DIEA (0.21 ml, 1.25 mmol) was added. The reaction mixture was stirred vigorously and Fmoc-D-Phe-Cl (0.507 g, 1.25 mmol) was added in five portions. Carpino, L. A. et al., *J. Org. Chem.* 51:3732-3734 (1986). Stirring was continued for 1 hour at 0 °C and then 24 hours at room temperature. The solvent was removed and the residue taken up with ethyl acetate (30 ml), washed with 0.1 N KHSO₄ (3x10 ml), saturated with aqueous NaCl solution (3x10 ml), dried over Na₂SO₄; and evaporated to dryness. The residue was purified by flash chromatography on silica gel 60 (chloroform/methanol 94/6). Still W. C. et al. *J. Org. Chem.* 14:2923-2925 (1978). The fractions containing pure products were combined, evaporated and dried under vacuum.

Yield: 0.305 g (0.47 mmol) 47.0%
 $C_{39}H_{40}N_2O_4$ M.W. = 648.73 g/mol
 $[\alpha]_D: -3.5^\circ (c=1.0 \text{ MeOH})$
TLC Results: R_f :(S3) 0.77
 R_f :(S4) 0.86
HPLC Results: t_R 54.2 min
FAB-MS: 631 ((M-H₂O)⁻), 650 ((M+1)⁺)

Example 3: Fmoc-D-Phe-Ψ[CO-N(CH₂CH₂NHBoc)]Phe-OH

5 A suspension of HN(CH₂CH₂NHBoc)Phe-OH (0.308 g, 1.0 mmol) in DCM (10 ml) was treated with BTSA (0.98 ml, 4 mmol) at room temperature for 24 hours. The clear solution was cooled to 0 °C and DIEA (0.34 ml, 2.0 mmol) was added. The reaction mixture was stirred vigorously and Fmoc-D-Phe-Cl (0.507g, 1.25mmol) was added in five portions within 20 minutes. Carpino et al. *J. Org. Chem.* 51:3732-3734 (1986). Stirring
 10 was continued for 1 hour at 0 °C and then 24 hours at room temperature. The solvent was removed and the residue taken up with ethyl acetate (30 ml), washed with 0.1 N KHSO₄ (3x10 ml), saturated aqueous NaCl solution (3x10 ml), dried over Na₂SO₄; and evaporated to dryness. The residue was purified by flash chromatography on silica gel 60 (chloroform/methanol 92/8). Still et al. *J. Org. Chem.* 14:2923-2925 (1978). The fractions
 15 containing pure product were combined, evaporated and dried under vacuum to yield a pale yellow oil.

Yield: 0.293 g (0.43 mmol) 43.3%
 C₄₀H₄₃N₃O₇ M.W.= 677.77 g/mol
 [α]_D: -21.4°(c=1.0 MeOH)
 20 TLC Results: R_f(S3): 0.45
 R_f(S4): 0.52
HPLC Results: t_R: 49.4 min
FAB-MS: 578 ((M+1-Boc)⁺), 678 (M⁺), 716 ((M-1+K)⁺)

Example 4: Fmoc-ValΨ[CO-N(CH₂COOBu^t)]Phe-OH

25 A mixture of HN(CH₂COOBu^t)Phe-OH (2.0 g, 7.16 mmol) in DCM (100 ml) was treated with BTSA (7.00 ml, 28.64 mmol) at room temperature for 24 hours. The clear solution was cooled to 0 °C and DIEA (1.425 ml, 8.59 mmol) was added. The reaction mixture was stirred and Fmoc-Val-Cl (2.55 g, 7.16 mmol) was added in five portions.
 30 Stirring was continued for 1 hour at 0°C and then for 24 hours at room temperature. The solvent was removed and the residue taken up with ethyl acetate (100 ml), washed with 0.1M KHSO₄ (3x30 ml), saturated aqueous NaCl solution (3x30 ml), dried over Na₂SO₄; and evaporated to dryness. The residue was purified by flash chromatography on silica gel 60(chloroform/methanol 94/6). The fractions containing pure product were combined,
 35 evaporated and dried under vacuum.

Yield: 3.4 g (79%) (without flash chromatography)
 1.13 g (27%) (after flash chromatography)
 C₃₅H₄₀N₂O₇ M.W. 600.69

	<u>TLC Results:</u>	$R_f(S_3)$ 0.83
5		$R_f(S_4)$ 0.71
	<u>HPLC Results:</u>	t_R : 51.2 min
	<u>FAB-MS:</u>	601 (M+1) ⁺

Example 5: Fmoc-ValΨ[CO-N(CH₂-CH₂-CH₂-NHBoc)]Phe-OH

10 A suspension of HN(CH₂-CH₂-CH₂-NHBoc)]Phe-OH (322 mg, 1.0 mmol) in DCM (20 ml) was treated with N,O-bis(trimethylsilyl)acetamide (980 μl, 4.0 mmol) at room temperature for 20 hours. The clear solution was cooled to 0 °C and DIEA (340 μl, 2.0 mmol) was added. The reaction mixture was stirred and Fmoc-Val-Cl (446 mg, 1.25 mmol) added over the course of 20 minutes. Stirring was continued for 1 hour at 0 °C and then for
15 24 hours at room temperature. The solvent was removed and the residue taken up in ethyl acetate (100 ml), washed with 0.1M KHSO₄ (3x30 ml), saturated aqueous NaCl solution (3x30 ml), dried over Na₂SO₄; and evaporated to dryness. The crude product were purified by flash chromatography on silica gel 60 (chloroform/methanol 91/9). The fractions containing pure product were combined, evaporated and dried under vacuum to yield a pale
20 yellow oil.

Yield: 550 mg (85%) (crude product)

C₃₇H₄₅N₃O₇ M.W. 643.79

TLC Results: $R_f(S_2)$ 0.63

HPLC Results: t_R : 48.50 min

25 FAB-MS: 644 (M⁺)

Example 6: Fmoc-ValΨ[CO-N(CH₂-CH₂-NHBoc)]Phe-OH

A suspension of HN(CH₂-CH₂-NHBoc)]Phe-OH (925 mg, 3.0 mmol) in DCM (20 ml) was treated with N,O-bis(trimethylsilyl)acetamide (2.94 ml, 12 mmol) at room
30 temperature for 20 h. The clear solution was cooled to 0 °C and DIEA (1.02 ml, 6 mmol) was added. The reaction mixture was stirred and Fmoc-Val-Cl (1.34 g, 3.75 mmol) added in some portions within 20 min. Stirring was continued for 1 hour at 0 °C and 24 hours at room temperature. The solvent was removed and the residue taken up in ethyl acetate (100 ml), washed with 0.1M KHSO₄ (3x30 ml), saturated aqueous NaCl solution (3x30 ml), dried
35 over Na₂SO₄; and evaporated to dryness. The crude product were purified by flash chromatography on silica gel 60 (chloroform/methanol 91/9). The fractions containing pure product were combined, evaporated and dried under vacuum to yield a pale yellow oil.

Yield: 1.5 g (80%) (crude product)

	$C_{36}H_{43}N_3O_7$	M.W. 629.76
5	<u>TLC Results:</u>	$R_f(S_2)$ 0.72
	<u>HPLC Results:</u>	t_R : 48.36 min
	<u>FAB-MS:</u>	630 (M^+)

Example 7: Boc- β Ala $\Psi(CH_2-NH)Phe-OH$

- 10 Boc- β Ala $\Psi(CH_2-NH)Phe-Obzl$ (5.0 g, 12.12 mmol) was hydrogenated in aqueous acetic acid (90%, 50 ml) with Pd-acetate (250-500 mg) for 12 hours. The reaction was monitored by TLC in hexane-ethylacetate 1/1. The catalyst was filtrated through a layer of silica gel (Merck, 0.04-0.063 mm), and the palladium was washed black with acetic acid (3x30 ml). The filtrates were separately evaporated under vacuum to dryness. To remove
15 impurities, the substances was washed intensively with ether and water to catch the phenylalanine, and then evaporated to dryness with ether.

Yield: 2.5 g (64 %)
 $C_{17}H_{26}N_2O_4$ M.W. 322.4

TLC Results: $R_f(S_1)$ 0
20 $R_f(S_4)$ 0.28
 $R_f(S_5)$ 0.64
HPLC Results: t_R 14.01 (min)
FAB-MS: 323 ($M+1$)⁺

25 **Example 8: Fmoc-Val $\Psi[CH_2-N(CO-CH_2-CH_2-NHBoc)]Phe$
and Z-Val $\Psi[CH_2-N(CO-CH_2-CH_2-NHBoc)Phe-Obzl$**

- Z-Val $\Psi(CH_2-NH)Phe-Obzl$ (2.5 g, 4.9 mmol) were dissolved in 20 ml DMF and at 0 °C added consecutively with Boc- β Ala (1.85 g, 9.8 mmol), HATU (3.72 g, 9.8 mmol), HOBT (1.33 g, 9.8 mmol) and DIEA (3.24 ml, 19.6 mmol). The reaction solution was
30 stirred 1 hour at 0 °C and overnight at room temperature. The solvent was removed under vacuum and the residue dissolved in 200 ml ethylacetate to provide a solution which was then washed with 0.1 M $KHSO_4$, with 0.1 M $NaHCO_3$ and with water. After drying over Na_2SO_4 , the ethylacetate was removed.

- The resulting solid residue (3 g) contained both pseudodipeptide and Boc- β Ala, and
35 so the crude product was recoupled with PyBrop. The reaction product was resolved in 120 ml CH_2Cl_2 and added to Boc- β Ala (1.85 g), PyBrop (4.56 g, 9.8 mmol), and DIEA (4.86 ml, 29.6 mmol) at 0°C. The reaction mixture was stirred for 72 hours at room temperature. After the solvent was removed, the residue was dissolved in 200 ml ethylacetate and again

extracted as described above. After the ethylacetate was removed, the crude reaction product
 5 was dissolved in 60 ml methanol and precipitated with 0.6% aqueous acetic acid. The precipitate shows a purity sufficient for further use in the following steps.

Yield: not determined
 $C_{37}H_{47}N_3O_7$ M.W. 645.77
 F. 183-185°C
 10 $[\alpha]^{20} = -2.7 \pm 1$ (c= 1.0, CH₃OH)
 $E_A(\text{Arg}) = 0$
TLC Results:
 $R_f(S_2)$ 0.9
 $R_f(S_4)$ 0.75
 $R_f(S_5)$ 0.85
 15 $R_f(S_6)$ 0.8
HPLC Results: t_R : 53.5 min
FAB-MS: $[M^+]$: obsd. 646

Example 9: H-ValΨ[CH₂-N(CO-CH₂-CH₂-NHBoc)]Phe-OH

20 Z-ValΨ[CH₂-N(CO-CH₂-CH₂-NHBoc)]Phe-Obzl (0.5 g) was dissolved in 50 ml acetic acid (95%) and hydrogenated in the presence of catalyst (palladium acetate). The catalyst was added in three portions. After hydrogenation overnight the catalyst was removed by filtration and the solution was evaporated to dryness. The triturated crude product was extracted with ethyl ether, dissolved in n-butanol and washed with 0.1 M
 25 KHSO₄. The butanol solution was washed with water and evaporated.

Yield: not determined
 $C_{22}H_{35}N_3O_5$ M.W. 421.51
 F. degradation
 $[\alpha] = +12.9 \pm 1$ (c=1.0, CH₃OH)
 30 $E_A(\text{Arg}) = 0$
TLC Results:
 $R_f(S_2)$ 0.6
 $R_f(S_4)$ 0.2
 $R_f(S_5)$ 0.55
 $R_f(S_6)$ 0.3
 35 HPLC Results: t_R : 31.4 min
FAB-MS: $[M^+]$: obsd. 422

Example 10: HN(CH₂-CH₂-COOAlI)Phe-OBu'

5 A solution of formyl Meldrum's acid (6.0g, 34.8mmol) and allylic alcohol (2.85ml, 41.8mmol) in dry toluene (60ml) was refluxed for 1.5 hours. The solvent was evaporated under vacuum and the crude product used without further purification.

The crude product was dissolved in absolute methanol (30ml) and added to a solution of H-Phe-OBu'-HAc (9.79g, 34.8mmol) and triethylamine (5.4ml, 34.8mmol) in dry
 10 methanol (30ml) at 0 °C. The solution was stirred with 3 g molecular sieves at room temperature for 1 hour, and then NaCNBH₃ (2.4g, 38.3mmol) was added at 0 °C. Stirring continued at room temperature for 12 hours. The reaction mixture was then filtered and the solvent was removed. The residue was taken up with ethyl acetate (200ml), washed with 5% KHSO₄ (6 x 600ml), 5% NaHCO₃ (3 x 100ml), saturated aqueous NaCl solution (3 x
 15 100ml), dried over Na₂SO₄; and evaporated to dryness to yield a white powder.

Yield: 7.1g

C₁₉H₂₇NO₄ MW: 333.42

TLC Results: R_F: 0.82 (hexane/ethyl acetate 1/1 v/v)

HPLC Results: t_R: 27.7 min

20 FAB-MS: 334 (M+H)

Example 11: HN(CH₂-CH₂-CH₂-NH-Alloc)Phe-OBu'

18.0g (83.24mmol) Alloc-_{Ala}-N,O-dimethylhydroxamate was dissolved in 200ml dry ether and cooled to 0°C. To the clear solution 3.9g (103.9mmol) LiAlH₄ was added
 25 carefully in small portions. The reaction mixture was stirred for 10 minutes at 0 °C and 40 minutes at room temperature. The reaction was finished with KHSO₄ (19.8g, 145.7mmol) in 100ml water at 0 °C. The ether phase was separated and the suspension of LiAlH₄/KHSO₄ was washed 3 times with ether (50ml). The ether phase was extracted with KHSO₄ (3 x 30ml), NaHCO₃ (3 x 50ml) and NaCl (3 x 50ml). The aldehyde (oil) was used as crude
 30 product. Yield: 8.0g

Phe-OBu'-HAc (14.3g, 50.8mmol) was dissolved in dry methanol (50ml) cooled to 0 °C, triethylamine (7.0ml, 50.8mmol) and Alloc- β Ala-CHO (8.0g, 50.8mmol) in 50ml methanol and molecular sieve (2-3 g) were added. The reaction mixture was stirred for 1 hour at room temperature, cooled to 0 °C and then NaCNBH₃ (3.52g, 55.9mmol) was added
 35 in small portions. The stirring was continued for 12 hours at room temperature. After removing the molecular sieves, the solvent was evaporated, the residue was taken up with ethyl acetate (150ml), washed with KHSO₄ 3 x 30ml, NaHCO₃ (3 x 50ml) and NaCl (3 x

50ml). The organic phase was dried over Na_2SO_4 and the solvent removed by evaporation.

- 5 The crude product (17g) was washed with ether and hexane to get a white powder.

Yield: 7.8g

$\text{C}_{20}\text{H}_{30}\text{N}_2\text{O}_4$ MW: 362.46

TLC Results: R_f : 0.44 (hexane/ethyl acetate 1/1 v/v)

0.58 (chloroform/methanol 9/1 v/v)

10 0.84 (n-butanol/HAc/water 4/1/1 v/v/v)

HPLC Results: t_R : 27.14 min

FAB-MS: 363 (M+H)

Example 12: Fmoc-DPhe [CO-N(CH₂-CH₂-CH₂-NHAlloc)]Phe-OH

- 15 To a solution of $\text{NH}(\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-NHAlloc})\text{Phe-OBu}^t$ (0.8g, 2.2mmol) in dry DCM were mixed 440 μl collidine (3.3 mmol) and 1.8g Fmoc-DPhe-F (4.4mmol).

The clear solution was stirred by 50 °C over 4 hours. The solvent was removed under vacuum and the crude product was resolved in ethyl acetate and extracted with 5% KHSO_4 (3x30ml), NaHCO_3 (3x30ml) and NaCl (3x30ml). The dry organic layer was evaporated
20 under vacuum.

Fmoc-DPhe-[CO-N(CH₂-CH₂-CH₂-NHAlloc)]Phe-OBu^t was purified by flash chromatography on silica gel 60 (MERCK) with pure chloroform. The fractions without Fmoc-DPhe-OH were collected and evaporated under vacuum. The resulting oil was treated with 50% TFA/DCM (and some drops of water) for 2 hours at room temperature. The
25 product (1.0g) was obtained as an oil and purified by flash chromatography on silica gel with chloroform /methanol 94/6.

Yield: 550mg (37%)

$\text{C}_{40}\text{H}_{41}\text{N}_3\text{O}_7$ MW: 675.75

TLC Results: R_f : 0.42 (chloroform/methanol 9/1 v/v)

30 HPLC Results: t_R 47.34 min

FAB-FAB-MS: 676 (M⁺)

35

Example 13: Fmoc-Val [CO-N(CH₂-CH₂-COOAll)]Phe-OH

5 To a mixed solution of NH(CH₂-CH₂-COOAll)Phe-OBu^t (700 mg, 2.1 mmol) in DCM, 417 µl collidine (3.15mmol) and 1.5 g Fmoc-Val-F (4.12 mmol) were added. The clear solution was stirred at 50 °C bath temperature for 4 hours. The reaction mixture was extracted with KHSO₄ (3 x 30 ml), NaHCO₃ (3 x 30ml) and NaCl (3 x 30ml). The dried organic phase was evaporated under vacuum, and the resulting oil was treated with

10 TFA/DCM 1:1 (and some drops of water) for 2 hours at room temperature. The TFA/DCM-solution was evaporated under vacuum and washed 3 times with ether. The crude product was obtained as an oil and purified by flash chromatography on silica gel 60 (MERCK) system 92/8 chloroform/methanol.

Yield: 1.96 g

15 $C_{35}H_{38}N_2O_7$ MW: 598.67

$[\alpha]_D^{25}$: -75₋₁°(c=1, MeOH)

TLC Results: R_F: 0.46 (chloroform/methanol 9/1 v/v)

HPLC Results: t_R 48.35 min

FAB-MS: 599.6 (M+H)

20

Example 14: Fmoc-DVal [CO-N(CH₂-CH₂-COOAll)]Phe-OH

To a mixed solution of NH(CH₂-CH₂-COOAll)Phe-OBu^t (230 mg, 0.7 mmol) in DCM, 139 µl collidine (1.05 mmol) and 510 mg Fmoc-DVal-F (1.4mmol) were added. The clear solution was stirred at 50 °C bath temperature for 4 hours. The reaction mixture

25 was extracted with KHSO₄ (3 x 30 ml), NaHCO₃ (3 x 30ml) and NaCl (3 x 30ml). The dried organic phase was evaporated under vacuum and the resulting oil was treated with TFA/DCM 1:1 (and some drops of water) for 2 hours at room temperature. The TFA/DCM-solution was evaporated under vacuum and washed 3 times with ether. The crude product was an oil which was purified by flash chromatography on silica gel 60 (MERCK) system

30 93/7 chloroform/methanol.

Yield: 523 mg

$C_{35}H_{38}N_2O_7$ MW: 598.67

$[\alpha]_D^{25}$: -34₋₁°(c=1, MeOH)

TLC Results: R_F: 0.42 (chloroform/methanol 9/1 v/v)

35 HPLC Results: t_R 47.76 min

FAB-MS: 599.9 (M+H) 597.5 (M-H)

Example 15: Fmoc-DVal [CO-N(CH₂-CH₂-CH₂-NHAlloc)Phe-OH

5 To a mixed solution of NH(CH₂-CH₂-CH₂-NHAlloc)Phe-OBu^t (200 mg, 0.56 mmol) in DCM, 100 µl collidine (0.84 mmol) and 400 mg Fmoc-DVal-F (1.12mmol) were added. The clear solution was stirred at 50 °C bath temperature for 4 hours. The reaction mixture was extracted with KHSO₄ (3 x 30 ml), NaHCO₃ (3 x 30ml) and NaCl (3 x 30ml). The dried organic phase was evaporated under vacuum and the resulting oil was treated with

10 TFA/DCM 1:1 (and some drops of water) for 2 hours at room temperature. The TFA/DCM-solution was evaporated under vacuum and washed 3 times with ether. The crude product was obtained as an oil and was purified by flash chromatography on silica gel 60 (MERCK) system 94/6 chloroform/methanol.

Yield: 560 mg (mmol).

15 C₃₆H₄₁N₃O₇ MW: 627.71

TLC Results: R_F: 0.57 (chloroform/methanol 9/1 v/v)

HPLC Results: t_R 45.47 min

FAB-MS: 5628.8 (M+H) 626.8 (M-H)

Example 16: Fmoc-Val [CO-N(CH₂-CH₂-CH₂-NHAlloc)Phe-OH

20 To a mixed solution of NH(CH₂-CH₂-CH₂-NHAlloc)Phe-OBu^t (2.1 g, 5.88 mmol) in DCM, 1.19 ml collidine (8.82 mmol) and 4.2 g Fmoc-Val-F (11.8mmol) were added. The clear solution was stirred at 50 °C bath temperature for 4 hours. The reaction mixture was extracted with KHSO₄ (3 x 30 ml), NaHCO₃ (3 x 30ml) and NaCl (3 x 30ml). The dried

25 organic phase was evaporated under vacuum to yield an oil, which was then treated with TFA/DCM 1:1 (and some drops of water) for 2 hours at room temperature. The TFA/DCM-solution was evaporated under vacuum and washed 3 times with ether. The crude product was obtained as an oil, which was purified by flash chromatography on silica gel 60 (MERCK), system chloroform/methanol 92/8.

30 Yield: 5.85 g

C₃₆H₄₁N₃O₇ MW: 627.71

TLC Results: R_F: 0.70 (chloroform/methanol 9/1 v/v)

HPLC Results: t_R 47.29 min

FAB-MS: 628.6 (M+H)

Example 17: Fmoc-Val [CO-N(CH₂-CH₂-COOAll)]DPhe-OH

To a solution of NH(CH₂-CH₂-COOAll)DPhe -OBu^t (1.0g, 3.0mmol) in dry DCM were mixed 597µl collidine (4.5 mmol) and 2.15g Fmoc-Val-F (6.0mmol). The clear

solution was stirred at 50 °C for 4 hours. The solvent was then removed under vacuum and
 5 the crude product was resolved in ethyl acetate and extracted with solution of 5% KHSO₄
 (3x30ml), saturated NaHCO₃ (3x30ml), and saturated NaCl (3x30ml). The dried (Na₂SO₄)
 organic layer was evaporated under vacuum.

Fmoc-Val-[CO-N(CH₂-CH₂-COOAl)]DPhe-OBu^t was purified by flash
 chromatography on silica gel 60 (MERCK) with pure chloroform. The fractions without
 10 Fmoc-Val-OH were collected and evaporated under vacuum. The resulting oil was treated
 with 50 % TFA/DCM (and some drops of water) for 2hour at room temperature. The crude
 product (1.0g) was obtained as an oil, which was purified by flash chromatography on silica
 gel with chloroform /methanol 96/4.

Yield: 255 mg
 15 C₃₅H₃₈N₂O₇ MW: 598.67
 [α]_D²⁵ +30.68° _ 1 (c=1, MeOH)
TLC Results: R_F: 0.79 (CH₂Cl/methanol 9/1 v/v)
 HPTLC-plates (Silica gel 60 F₂₅₄, Merck HPTLC-glass plates)
HPLC Results: t_R: HPLC 47.11 min
 20 (Knauer, 220 nm, HD-SIL 18-5s-80, 250x4 mm, gradient from 20%
 acetonitrile containing 0.1% TFA to 80% acetonitrile, flow rate 1
 ml/min)
FAB-FAB-MS: 599 (M⁻)

25

Example 18: Synthesis of Fmoc-Val-Hydroxamate

One equivalent of Fmoc-Val-OH (10.00 mmol; 3.4 g) and 1 equivalent N,O-
 dimethylhydroxylamine hydrochloride (10.00 mmol; 1.08 g) were dissolved in 100 ml THF.
 The mixture as cooled to 0 °C, at which point 1.1 equivalent DIEA (11.00 mmol; 1.88 ml)
 30 and 1 equivalent N,N'-dicyclohexylcarbodiimid (10.00 mmol; 2.06 g) were added and
 stirring was continued at 0 °C for 1 hour, and then at room temperature for 4 hours. The
 precipitating dicyclohexyl urea was filtered off and the solvent was removed under vacuum.
 The residue was dissolved in ethyl acetate and extracted with KHSO₄ (3x), NaHCO₃ (3x)
 35 and NaCl (3x). After drying with Na₂SO₄ and concentrating under vacuum, a wax-like
 substance was obtained. Remaining dicyclohexyl urea was separated using diethyl ether.
 The urea was filtered off, and the solvent removed under vacuum.

5	<u>Yield:</u>	93%
		$C_{22}H_{26}N_2O_4$ M_w 382 g/mol
	<u>TLC Results:</u>	R_F 0.14 (hexane/ ethyl acetate 8:2) R_F 0.76 (benzene/acetone/acetic acid 27:10:0.5) R_F 1 (chloroform/ methanol 9:1)
	<u>HPLC Results:</u>	t_R 38.10 (20-80% B in 60 min; Vydac C18)
10	<u>FAB-MS:</u>	$[MH]^+$ 383

Example 19: Synthesis of Fmoc-Val-CHO

One equivalent Fmoc-Val-hydroxamate (13.07 mmol; 5.00 g) was dissolved in dry THF. This solution was evacuated and covered with argon. The mixture was then cooled to -15°C and 1.25 equivalents $LiAlH_4$ (16.33 mmol; 0.62 g) was carefully added in portions. Stirring was continued at -15°C for 15 min and then at room temperature for 1 hour. The mixture was then hydrolyzed with a concentrated solution of 1.75 equivalents $KHSO_4$ (22.87 mmol; 3.11 g), and the THF was evaporated under vacuum. The remaining aqueous solution was extracted with diethyl ether several times. The extracts were combined and extracted with $KHSO_4$ (3x), $NaHCO_3$ (3x) and $NaCl$ (3x). After drying with Na_2SO_4 and concentrating under vacuum, an oil was obtained that was immediately used for further processing.

25	<u>Yield:</u>	60-80%
		$C_{20}H_{21}NO_3$ M_w 323 g/mol
	<u>TLC Results:</u>	R_F 0.37 (hexane/ ethyl acetate 8:2) R_F 0.89 (benzene/acetone/ acetic acid 27:10:0.5) R_F 1 (chloroform/ methanol 9:1)
30	<u>HPLC Results:</u>	t_R 36.26 (20-80% B in 60 min; Vydac C18)
	<u>FAB-MS:</u>	$[MH]^+$ 324

Example 20: Synthesis of Fmoc-Val Ψ [CH $_2$ NH]Phe-OBu'

One equivalent Phe-OBu'xHAc (30.95 mmol; 8.72 g) was treated with 1 equivalent triethylamine (30.95 mmol; 4.31 ml) and was then added to a solution of 1 equivalent Fmoc-Val-CHO (30.95 mol; 10.00 g) in dry methanol with molecular sieves. This mixture was stirred for 1 hour before it was cooled to 0 °C, at which point 1.1 equivalent $NaCNBH_3$ (34.05 mmol; 2.14 g) was added in portions. Stirring was continued overnight. The

molecular sieves were filtered off and the methanol removed under vacuum to provide a
 5 residue which was then dissolved in ethyl acetate and extracted with KHSO_4 (3x), NaHCO_3 (3x) and NaCl (3x). After drying with Na_2SO_4 and concentrating under vacuum, a white solid compound was obtained.

Yield: 83% (crude product)

$\text{C}_{33}\text{H}_{40}\text{N}_2\text{O}_4$ M_w 528 g/mol

10 TLC Results: R_F 0.25 (hexane/ ethyl acetate 8:2)
 R_F 0.57 (chloroform/ methanol 9:1)
 R_F 0.88 (benzene/ acetone/ acetic acid 27:10:0.5)

HPLC Results: t_R 42.21 (20-80% B in 60 min Vydac C18)

FAB-MS: $[\text{MH}]^+ 529$

15

Example 21: Synthesis of Fmoc-Val Ψ [CH $_2$ N(COCH $_2$ CH $_2$ NHAlloc)]Phe-OBu t

One equivalent Alloc- β -Ala (2 mmol; 0.346 g) was dissolved in dry THF and cooled to -15°C . One equivalent N-methyl morpholine (2 mmol; 0.22 ml) and 1 equivalent isobutyl chloroformate (2 mmol; 0.264 ml) were then added. After 10 minutes, a solution of 1
 20 equivalent Fmoc-Val Ψ [CH $_2$ NH]Phe-OBu t (2 mmol; 1.056 g) and 1 equivalent N-methyl morpholine (2 mmol; 0.22 ml) in dry THF was added. The mixture was allowed to warm up gently to room temperature. After stirring overnight, the THF was removed and the residue dissolved in ethyl acetate, extracted with KHSO_4 (3x), NaHCO_3 (3x) and NaCl (3x). The organic phase was dried with Na_2SO_4 , and the solvent removed under vacuum to provide a
 25 yellow oil.

Yield: 92% (crude product) about 35% content

$\text{C}_{40}\text{H}_{49}\text{N}_3\text{O}_7$ M_w 683 g/mol

TLC Results: R_F 0.15 (hexane/ ethyl acetate 8:2)
 R_F 0.86 (chloroform/ methanol 9:1)
 30 R_F 1.00 (benzene/acetone/acetic acid 27:10:0.5)

HPLC Results: t_R 55.11 (20-80% B in 60 min Vydac C18)

FAB-MS: $[\text{MH}]^+ 684$

Example 22: Synthesis of Fmoc-Val Ψ [CH $_2$ N(COCH $_2$ CH $_2$ NHAlloc)]Phe-OH

35 Fmoc-Val Ψ [CH $_2$ N(COCH $_2$ CH $_2$ NHAlloc)]Phe-OBu t (2.82 g) was treated with 90% TFA/water for 1 hour. After removing the TFA, the residue was washed with diethyl ether several times and then purified by flash-chromatography with chloroform/methanol (9:1) using silica gel 60 for column chromatography (particle size 0.040-0.063 mm).

	<u>Yield:</u>	23.5% (662 mg)	
5		$C_{36}H_{41}N_3O_7$	M_w 627 g/mol
		$[\alpha_D]^{25}$	- 51.6° (c=1; methanol)
		<u>TLC Results:</u>	
		R_F	0 (hexane/ethyl acetate 8:2)
		R_F	0.18 (chloroform/methanol 9:1)
		R_F	0.28 (benzene/acetone/acetic acid 27:10:0.5)
10	<u>HPLC Results:</u>	t_R	42.46 (20-80% B in 60 min Vydac C18)
	<u>FAB-MS:</u>	$[MH]^+$ 628	

Example 23: Synthesis of Fmoc-ValΨ[CH₂N(COCH₂CH₂COOAlI)]Phe-OBu^t

Succinic anhydride (0.2 mol; 20.00 g), allyl alcohol (0.6 mol; 12.30 ml) and a catalytic amounts of DMAP were combined in 200 ml toluene and refluxed for 4 hours. The solvent and excessive allyl alcohol were removed by evaporation and the crude product was fractionated under vacuum.

	<u>Yield:</u>	84%	
		$C_7H_{10}O_4$	M_w 158 g/mol
	<u>K_p:</u>	101°C at 0.072 mbar	
20	<u>FAB-MS:</u>	$[MH]^+$ 159	

One equivalent succinic acid monoallylester (2 mmol; 0.316 g) was dissolved in dry THF and cooled to -15°C. One equivalent N-methyl morpholine (2 mmol; 0.22 ml) and 1 equivalent isobutyl chloroformate (2 mmol; 0.264 ml) were then added. After 10 minutes, a solution of 1 equivalent Fmoc-ValΨ[CH₂NH]Phe-OBu^t (2 mmol; 1.056 g) and 1 equivalent N-methyl morpholine (2 mmol; 0.22 ml) in dry THF was added. The mixture was allowed to warm up gently to room temperature. After stirring overnight, the THF was removed and the residue dissolved in ethyl acetate, extracted with KHSO₄ (3x), NaHCO₃ (3x) and NaCl (3x). The organic phase was dried with Na₂SO₄ and the solvent was removed under vacuum to provide a yellow oil.

	<u>Yield:</u>	95% (crude product) about 20% content	
		$C_{40}H_{39}N_3O_7$	M_w 659 g/mol
	<u>TLC Results:</u>	R_F	0.31 (hexane/ ethyl acetate 8:2)
		R_F	0.93 (chloroform/ methanol 9:1)
35		R_F	1.00 (benzene/ acetone/ acetic acid 27:10:0.5)
	<u>HPLC Results:</u>	t_R	58.54 (20-80% B in 60 min Vydac C18)
	<u>FAB-MS:</u>	$[MH]^+$ 660	

Example 25: Synthesis of Fmoc-ValΨ[CH₂N(COCH₂CH₂COOAlI)]Phe-OH

5 Fmoc-ValΨ[CH₂N(COCH₂CH₂COOAlI)]Phe-OBu^t (2.40 g) was treated with 90% TFA/water for 1 hour. After removing the TFA, the residue was washed with diethyl ether several times and then purified by flash-chromatography with chloroform/methanol (9:1) using silica gel 60 for column chromatography (particle size 0.040-0.063 mm).

Yield: 26% (583 mg)

10 C₃₆H₄₀N₂O₇ 612 g/mol

[α_D]²⁵ -49.9° (c=1; methanol)

TLC Results: R_F 0 (hexane/ethyl acetate 8:2)

R_F 0.18 (chloroform/methanol 9:1)

R_F 0.28 (benzene/acetone/acetic acid 27:10:05)

15 HPLC Results: t_R 45.25 (20-80% B in 60 min Vydac C18)

FAB-MS: [MH]⁺ 613

Example 26: Synthesis of Fmoc-DPhe-Hydroxamate

One equivalent Fmoc-DPhe-OH (25.80 mmol; 10.00 g) and 1 equivalent N,O-
20 dimethylhydroxylamine hydrochloride (28.38 mmol; 2.78 g) were dissolved in 100 ml THF, and the mixture was cooled to 0°C. To this were added 1.1 equivalents DIEA (28.38 mmol; 4.86 ml) and 1 equivalent N,N'-dicyclohexylcarbodiimide (25.80 mmol; 5.32 g), and stirring was continued at 0 °C for 1 hour and then at room temperature for 4 hours. The precipitating dicyclohexyl urea was filtered off and the solvent was removed under vacuum. The residue
25 was dissolved in ethyl acetate and extracted with KHSO₄ (3x), NaHCO₃ (3x) and NaCl (3x). After drying with Na₂SO₄ and concentrating under vacuum, a wax-like substance was obtained. Remaining dicyclohexyl urea was separated using diethyl ether. The urea was filtered off and the solvent removed under vacuum.

Yield: 93%

30 C₂₆H₂₆N₂O₄ M_w 430 g/mol

TLC Results: R_F 0.09 (hexane/ ethyl acetate 8:2)

R_F 0.86 (benzene/acetone/acetic acid 27:10:0.5)

R_F 1 (chloroform/ methanol 9:1)

HPLC Results: t_R 40.52 (20-80% B in 60 min Vydac C18)

35 FAB-MS: [MH]⁺ 431

Example 27: Synthesis of Fmoc-DPhe-CHO

5 One equivalent Fmoc-DPhe-hydroxamate (23.23 mmol; 10.00 g) was dissolved in dry THF, and the solution was evacuated, covered with argon, and cooled to -15°C. 1.25 equivalents LiAlH₄ (29.04 mmol; 1.11 g) were then carefully added in portions. Stirring was continued at -15°C for 15 min and then at room temperature for 1 hour. The mixture was then hydrolyzed with a concentrated solution of 1.75 equivalent KHSO₄ (40.65 mmol; 10 5.53 g). The THF was evaporated under vacuum, and the remaining aqueous solution was extracted with diethyl ether several times. The extracts were combined and extracted with KHSO₄ (3x), NaHCO₃ (3x) and NaCl (3x). After drying with Na₂SO₄ and concentrating under vacuum, an oil was obtained that was immediately used for further processing.

Yield: 60-80%

15 $C_{24}H_{21}NO_3$ M_w 355 g/mol

TLC Results: R_F 0.16 (hexane/ ethyl acetate 8:2)
 R_F 0.80 (benzene/acetone/acetic acid 27:10:0.5)
 R_F 1 (chloroform/ methanol 9:1)

HPLC Results: t_R 35.57 (20-80% B in 60 min Vydac C18)

20 FAB-MS: $[MH]^+$ 356

Example 28: Synthesis of Fmoc-DPheΨ[CH₂NH]Phe-OBu^t

One equivalent Phe-OBu^txHAc (11.42 mmol; 3.22 g) was treated with 1 equivalent triethylamine (11.42 mmol; 1.60 ml) and then added to a solution of 1 equivalent Fmoc-
 25 DPhe-CHO (11.42 mmol; 4.06 g) in dry methanol with molecular sieves. This mixture was stirred for 1 hour before being cooled to 0 °C. 1.1 equivalents NaCNBH₃ (12.56 mmol; 0.70 g) were added in portions, and stirring was continued overnight. The molecular sieves were filtered off and methanol evaporated under vacuum. The residue was dissolved in ethyl acetate and extracted with KHSO₄ (3x), NaHCO₃ (3x) and NaCl (3x). After drying with
 30 Na₂SO₄ and concentrating under vacuum, a yellow oil was obtained.

Yield: 98% (crude product) 41% content

$C_{37}H_{40}N_2O_4$ M_w 576 g/mol

TLC Results: R_F 0.21 (hexane/ ethyl acetate 8:2)
 R_F 0.89 (benzene/acetone/acetic acid 27:10:0.5)
 35 R_F 0.73 (chloroform/ methanol 9:1)

HPLC Results: t_R 43.98 (20-80% B in 60 min Vydac C18)

FAB-MS: $[MH]^+$ 577

Example 29: Synthesis of Fmoc-DPheΨ[CH₂N(COCH₂CH₂NHAlloc)]Phe-OBu'

5 One equivalent Alloc-β-Ala (2 mmol; 0.346 g) is dissolved in dry THF and cooled to -15°C. One equivalent N-methyl morpholine (2 mmol; 0.22 ml) and 1 equivalent isobutyl chloroformate (2 mmol; 0.264 ml) were then added. After 10 minutes, a solution of 1 equivalent Fmoc-DPheΨ[CH₂NH]Phe-OBu' (2 mmol; 1.152 g) and 1 equivalent N-methyl morpholine (2 mmol; 0.22 ml) in dry THF was added. The mixture was allowed to gently
 10 warm up to room temperature. After stirring overnight, the THF was removed and the residue dissolved in ethyl acetate, extracted with KHSO₄ (3x), NaHCO₃ (3x) and NaCl (3x). The organic phase was dried with Na₂SO₄ and the solvent removed under vacuum to provide a yellow oil.

Yield: 96% (crude product) about 31% content

15 $C_{44}H_{49}N_3O_7$ M_w 731 g/mol

TLC Results:

R_F	0.09	(hexane/ ethyl acetate 8:2)
R_F	0.85	(chloroform/ methanol 9:1)
R_F	1.00	(benzene/acetone/acetic acid 27:10:0.5)

HPLC Results: t_R 55.26 (20-80% B in 60 min Vydac C18)

20 FAB-MS: $[MH]^+$ 732

Example 30: Synthesis of Fmoc-DPheΨ[CH₂N(COCH₂CH₂NHAlloc)]Phe-OH

Fmoc-DPheΨ[CH₂N(COCH₂CH₂NHAlloc)]Phe-OBu' (2.275 g) is treated with 90% TFA/water for 1 hour. After removing the TFA, the residue was washed with diethyl ether
 25 several times and purified by flash-chromatography with chloroform/methanol (9:1) using silica gel 60 for column chromatography (particle size 0.040-0.063 mm).

Yield: 27.5% (577 mg)

$C_{40}H_{41}N_3O_7$ M_w 675 g/mol

$[\alpha_D]^{25}$ - 32.6° (c=1; methanol)

30 TLC Results:

R_F	0	(hexane/ethyl acetate 8:2)
R_F	0.44	(chloroform/methanol 9:1)
R_F	0.35	(benzene/acetone/acetic acid 27:10:0.5)

HPLC Results: t_R 43.25 (20-80% B in 60 min Vydac C18)

FAB-MS: $[MH]^-$ 676

35

Example 31: Synthesis of Fmoc-DPheΨ[CH₂N(COCH₂CH₂COOAll)]Phe-OBu'

5 Succinic anhydride (0.2 mol; 20.00 g), allyl alcohol (0.6 mol; 12.30 ml) and a catalytic amounts of DMAP in 200 ml toluene were refluxed for 4 hours. The solvent and excessive allyl alcohol were removed and the crude product fractionated under vacuum.

Yield: 84%

C₇H₁₀O₄ M_w 158 g/mol

10 K_p: 101°C at 0.072 mbar

FAB-MS: [MH]⁺ 159

One equivalent succinic acid monoallylester (2 mmol; 0.316 g) was dissolved in dry THF and cooled to -15°C. One equivalent N-methyl morpholine (2 mmol; 0.22 ml) and 1
15 equivalent isobutyl chloroformate (2 mmol; 0.264 ml) are added. After 10 min a solution of 1 equivalent Fmoc-DPheΨ[CH₂NH]Phe-OBu' (2 mmol; 1.152 g) and 1 equivalent N-methyl morpholine (2 mmol; 0.22 ml) in dry THF were then added. The mixture was allowed to warm up gently to room temperature. After stirring overnight, the THF was removed and the residue was dissolved in ethyl acetate, extracted with KHSO₄ (3x), NaHCO₃ (3x) and
20 NaCl (3x). The organic phase is dried with Na₂SO₄ and the solvent was removed under vacuum to provide a yellow oil.

Yield: 92% (crude product) about 27% content

C₄₄H₄₈N₂O₇ M_w 716 g/mol

TLC Results: R_F 0.20 (hexane/ ethyl acetate 8:2)

25 R_F 0.93 (chloroform/ methanol 9:1)

R_F 1.00 (benzene/ acetone/ acetic acid 27:10:0.5)

HPLC Results: t_R 58.67 (20-80% B in 60 min Vydac C18)

FAB-MS: [MH]⁺ 717

30 **Example 32: Synthesis of Fmoc-DPheΨ[CH₂N(COCH₂CH₂COOAll)]Phe-OH**

Fmoc-DPheΨ[CH₂N(COCH₂CH₂COOAll)]Phe-OBu' (2.19 g) was treated with 90% TFA/water for 1 hour. After removing the TFA, the residue was washed with diethyl ether several times and purified by flash-chromatography with chloroform/methanol (9:1) using silica gel 60 for column chromatography (particle size 0.040-0.063 mm).

35 Yield: 36% (727 mg)

C₄₀H₄₀N₂O₇ M_w 660 g/mol

[α_D]²⁵ - 23.5° (c=1; methanol)

TLC Results: R_F 0 (hexane/ethyl acetate 8:2)

5	R_F	0.48	(chloroform/methanol 9:1)
	R_F	0.52	(benzene/acetone/acetic acid 27:10:0.5)
	<u>HPLC Results:</u>	t_R	46.00 (20-80% B in 60 min Vydac C18)
	<u>FAB-MS:</u>	[MH] ⁺ 661	

10 While the present invention has been described with respect to the particular
embodiments and examples provided above, it will be apparent to those skilled in the art
that various changes and modifications may be made without departing from the spirit and
scope of the invention as defined in the claims. Such modifications are intended to fall
within the scope of the appended claims.

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THE CLAIMS

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1. A building unit of Formula (I):



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wherein AA_m and AA_p are residues of natural or synthetic amino acids; U is $C^\alpha ON[(CH_2)_{n+1}W]$ or $\Psi(C^\alpha H_2 N)[CO(CH_2)_q W]$; C^α is a carbon atom and is the α carbon of AA_m if $m > 0$; W is NH-X or COY; X is a hydrogen atom of an amine or amide or an amine or amide protecting group; Y is the hydroxyl group of a carboxylic acid moiety or a
 15 carboxylic acid protecting group; m is a number from 0 to 10; n is a number from 0 to 6; p is a number from 1 to 10; and q is a number from 1 to 6.

2. The building unit of claim 1 wherein AA_m and AA_p each comprises one or more residues of an amino acid selected from the group consisting of D-Phe, Phe, D-Val, and Val; and Y is selected from the group consisting of hydroxyl, alkoxy, substituted
 20 alkoxy, allyloxy, substituted allyloxy, aryloxy, substituted aryloxy, and trimethylsilyl ether.

3. The building unit of claim 2 wherein X is selected from the group consisting of H, Boc, Z, Fmoc, and Alloc; and Y is selected from the group consisting of OMe, OEt, OBu^t, OBzl, and All.
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4. The building unit of claim 1, said building unit having the structure of Formula (II):



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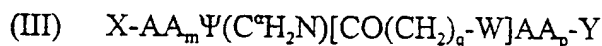
wherein n is a number from 0 to 4; m is a number from 0 to 4; and p is a number from 0 to 4.

35

- 5 5. The building unit of claim 4, said building unit being selected from the group consisting of:

HN(CH₂COOBu^t)Phe-OMe;
 Fmoc-D-Phe-[CO-N(CH₂COOBu^t)]Phe-OH;
 Fmoc-D-Phe-[CO-N(CH₂CH₂NHBoc)]Phe-OH;
 Fmoc-Val-[CO-N(CH₂COOBu^t)]Phe-OH;
 10 Fmoc-Val-[CO-N(CH₂-CH₂-CH₂-NHBoc)]Phe-OH;
 Fmoc-Val-[CO-N(CH₂-CH₂-NHBoc)]Phe-OH;
 Boc-βAla -(CH₂-NH)Phe-OH;
 Fmoc-DPhe-[CO-N(CH₂-CH₂-CH₂-NHAlloc)]Phe-OH;
 Fmoc-Val-[CO-N(CH₂-CH₂-COOAll)]Phe-OH;
 15 Fmoc-DVal-[CO-N(CH₂-CH₂-COOAll)]Phe-OH;
 Fmoc-DVal-[CO-N(CH₂-CH₂-CH₂-NHAlloc)]Phe-OH;
 Fmoc-Val-[CO-N(CH₂-CH₂-CH₂-NHAlloc)]Phe-OH; and
 Fmoc-Val-[CO-N(CH₂-CH₂-COOAll)]DPhe-OH.

- 20 6. The building unit of claim 1, said building unit having the structure of Formula (III):



wherein n is a number from 0 to 3; m is a number from 0 to 4; and p is a number from 0 to 4, and q is a number from 0 to 4.

7. The building unit of claim 6, said building unit being selected from the group consisting of:

Fmoc-ValΨ[CH₂-N(CO-CH₂-CH₂-NHBoc)]Phe;
 30 Z-ValΨ[CH₂-N(CO-CH₂-CH₂-NHBoc)]Phe-Obzl;
 H-ValΨ[CH₂-N(CO-CH₂-CH₂-NHBoc)]Phe-OH;
 Fmoc-ValΨ[CH₂NH]Phe-OBu^t;
 Fmoc-ValΨ[CH₂N(COCH₂CH₂NHAlloc)]Phe-OBu^t;
 Fmoc-ValΨ[CH₂N(COCH₂CH₂NHAlloc)]Phe-OH;
 35 Fmoc-ValΨ[CH₂N(COCH₂CH₂COOAll)]Phe-OBu^t;
 Fmoc-ValΨ[CH₂N(COCH₂CH₂COOAll)]Phe-OH;
 Fmoc-DPheΨ[CH₂N(COCH₂CH₂NHAlloc)]Phe-OBu^t;
 Fmoc-DPheΨ[CH₂N(COCH₂CH₂NHAlloc)]Phe-OH;

Fmoc-DPheΨ[CH₂N(COCH₂CH₂COOAll)]Phe-OBu^t; and

5 Fmoc-DPheΨ[CH₂N(COCH₂CH₂COOAll)]Phe-OH.

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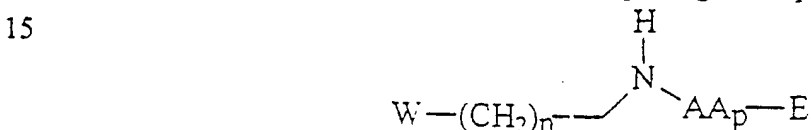
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- 5 8. A method of synthesizing a building unit of Formula (II):



wherein AA_m and AA_p are residues of natural or synthetic amino acids; C^{α} is the α carbon of
 10 AA_m if $m > 0$ or else is simply a carbon atom; W is $NH-X$ or COY ; X is a hydrogen atom of
 an amine or amide or an amine or amide protecting group; Y is the hydroxyl group of a
 carboxylic acid moiety or a carboxylic acid protecting group; m is a number from 0 to 10; n
 is a number from 0 to 6; and p is a number from 1 to 10; said method comprising:

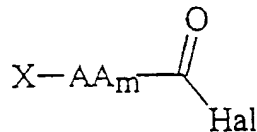
preparing a first reaction mixture comprising a compound of Formula (d):



Formula (d)

wherein E is a carboxyl protecting group and W , N and AA_p are as defined above; and

20 reacting said first reaction mixture with a compound of Formula (e):



Formula (e)

wherein Hal is a halogen and X and m are as defined above, under reaction conditions
 25 sufficient to form the building unit of Formula (II).

9. The method of claim 8 which further comprises selecting Hal to be F or Cl ;
 selecting X from the group consisting of H , Boc , Z , $Fmoc$, and $Alloc$; and selecting Y from
 the group consisting of OMe , OEt , OBu^t , $OBzl$, and All .

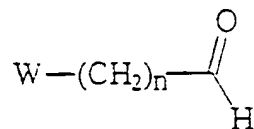
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10. The method of claim 8 or 9 which further comprises utilizing a catalyst to
 facilitate the reaction of the first reaction mixture with the compound of Formula (e), said
 catalyst being selected from the group consisting of collidine, $BSTA$, $HBTU$, $HOBT$, $DIEA$,
 and mixtures thereof.

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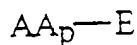
11. The method of claim 8 to 10 which further comprises removal of the
 protecting group E after formation of the building unit of Formula (II).

12. The method of any one of claims 8 to 11 wherein the first reaction mixture is prepared by reacting a compound of Formula (b):



Formula (b)

- 10 where W and n are as defined above, with a compound of Formula (c):



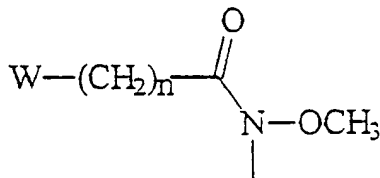
Formula (c)

where AA_p and E are as defined above, under reaction conditions sufficient to form the compound of Formula (d).

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13. The method of claim 12 which further comprises obtaining the compound of Formula (b) from cleavage of an N-alkoxyamide.

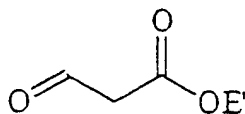
14. The method of claim 13 which further comprises selecting the N-alkoxyamide to be a compound of Formula (a):



Formula (a)

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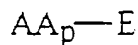
15. The method of any one of claims 8 to 11 wherein the first reaction mixture is prepared by reacting a compound of Formula (x):



Formula (x)

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wherein E' is a carbonyl protecting group, with a compound of Formula (c):

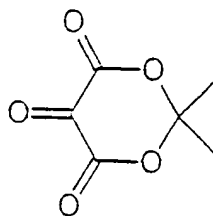


Formula (c)

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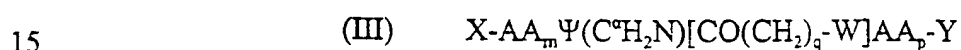
where AA_p and E are as defined above, under reaction conditions sufficient to form the compound of Formula (d).

16. The method of claim 15 wherein the compound of Formula (j) is prepared by
5 rearrangement of a compound of Formula (w):



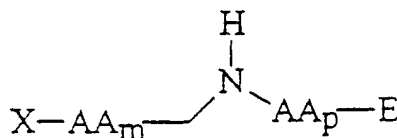
Formula (w)

17. A method of synthesizing a building unit of Formula (III):



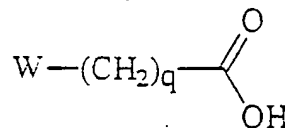
wherein AA_m and AA_p are residues of natural or synthetic amino acids; C^{α} is the α carbon of AA_m if $m > 0$ or else is simply a carbon atom; W is $NH-X$ or $COOY$; X is a hydrogen atom of an amine or amide or an amine or amide protecting group; Y is the hydroxyl group of a
20 carboxylic acid moiety or a carboxylic acid protecting group; m is a number from 0 to 10; p is a number from 1 to 10; and q is a number from 1 to 6; said method comprising:

preparing a first reaction mixture comprising a compound of Formula (m):



Formula (m)

wherein E is a carboxyl protecting group and X, AA_m and AA_p are as defined above; and
reacting said first reaction mixture with a compound of Formula (n):



Formula (n)

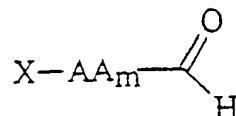
where W and q are as defined above, under reaction conditions sufficient to form the building unit of Formula (III).

18. The method of claim 17 which further comprises selecting X from the group
35 consisting of H, Boc, Z, Fmoc, and Alloc; and selecting Y from the group consisting of OMe, OEt, O*Bu*^t, OBzl, and All.

19. The method of claim 17 or 18 which further comprises utilizing a catalyst to
 5 facilitate the reaction of the first reaction mixture and the compound of Formula (n), said
 catalyst being selected from the group consisting of collidine, BSTA, HBTU, HOBT, DIEA,
 and mixtures thereof.

20. The method of any one of claims 17 to 19 which further comprises removal
 10 of the protecting group E after formation of the building unit of Formula (III).

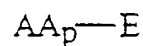
21. The method of any one of claims 17 to 20 wherein the first reaction mixture
 is prepared by reacting a compound of Formula (k):



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Formula (k)

where X and m are as defined above, with a compound of Formula (c):



Formula (c)

20 where AA_p and E are as defined above, under reaction conditions sufficient to form the
 compound of Formula (m).

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 5/02, 5/06, C07C 271/22, 271/20, 229/36, 237/10	A3	(11) International Publication Number: WO 99/31121 (43) International Publication Date: 24 June 1999 (24.06.99)
(21) International Application Number: PCT/EP98/08187 (22) International Filing Date: 14 December 1998 (14.12.98) (30) Priority Data: 08/990,591 15 December 1997 (15.12.97) US (71) Applicant (for all designated States except US): PEPTOR LTD. [IL/IL]; Kiryat Weizmann, 76326 Rehovot (IL). (72) Inventors; and (75) Inventors/Applicants (for US only): REISSMANN, Siegmund [DE/DE]; Werner-Seelenbinder-Strasse 14, D-07747 Jena (DE). MÜLLER, Bettina [DE/DE]; Erfurter Strasse 44, D-07743 Jena (DE). KLEINWÄCHTER, Peter [DE/DE]; Dr.-Theodor-Neubauer-Strasse 38, D-07546 Gera (DE). BESSER, Diana [DE/DE]; Jennergasse 13, D-07743 Jena (DE). (74) Agent: VOSSIUS & PARTNER; Siebertstrasse 4, D-81675 München (DE).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 19 August 1999 (19.08.99)
(54) Title: BRANCHED BUILDING UNITS FOR SYNTHESIZING CYCLIC PEPTIDES (57) Abstract <p>The present invention relates to building units suitable for the synthesis of backbone cyclized peptides. The building units of this invention have structures of the formula (I): $X-AA_m-U-AA_p-Y$ wherein AA_m and AA_p are residues of natural or synthetic amino acids and U is $C_\alpha ON[(CH_2)_{n+1}W]$ or $\Psi(C_\alpha H_2N)[CO(CH_2)_qW]$, in which C_α is a carbon atom and is the α carbon of AA_m if $m > 0$. W is $NH-X$ or COY, and X is H, Boc, Z, Fmoc, or Alloc while Y is the hydroxyl group of a carboxylic acid moiety or a carboxylic acid protecting group. The letter m refers to an integer from 0 to 10; n is a number from 0 to 6; p is a number from 1 to 10; and q is a number from 1 to 6. The present invention is also directed to methods of synthesizing building units of formula (I) via different routes.</p>		

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/08187

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K5/02 C07K5/06 C07C271/22 C07C271/20 C07C229/36
C07C237/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. BYK ET AL: "Building Units for N-backbone cyclic peptides. 1." J. ORG. CHEM., vol. 57, 1992, pages 5687-5692, XP002100997 cited in the application	1-4,6
Y	*Schemes I, IV, especially scheme V* see page 5690	8-21
X	--- G. BITAN ET AL.: "Building Units for N-backbone cyclic peptides. 2." TETRAHEDRON, vol. 51, no. 38, 1995, pages 10513-10522, XP002100998 *Scheme 5* --- -/--	1-4,6

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

23 April 1999

Date of mailing of the international search report

06/07/1999

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/08187

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D. MULLER ET AL.: "Building Units for N-backbone cyclic peptides. 3." J. ORG. CHEM., vol. 62, no. 2, January 1997, pages 411-416, XP002100999 *compound 11*	1-6
X	--- G. BITAN ET AL.: "Building Units for N-backbone cyclic peptides. 4." J. CHEM. SOC. PERKIN TRANSACTION 1, no. 10, May 1997, pages 1501-1510, XP002101000	1-4,6
Y	*Schemes 1-3*	8-21
X	--- EP 0 564 739 A (YISSUM RES DEV CO (IL))) 13 October 1993 see claims	1-4,6
X	--- WO 95 33765 A (PEPTOR LTD ;YISSUM RES DEV CO (IL); GILON CHAIM (IL); EREN DORON () 14 December 1995	1-4,6
Y	see page 41; claims	8-21
A	--- WO 97 09344 A (PEPTOR LIMITED ;YISSUM RES DEV CO (IL); HORNIK VERED (IL); GILON C) 13 March 1997	
A	--- KALJUSTE K ET AL: "A NEW GENERAL SOLID-PHASE METHOD FOR THE SYNTHESIS OF BACKBONE-TO- BACKBONE CYCLIZED PEPTIDES" INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH, vol. 43, no. 5, 1 May 1994, pages 505-511, XP000440871 cited in the application	
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 98/08187

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